



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>C12Q 1/68, C12N 5/10, 15/12, C07K 14/00, 16/18, G01N 33/53</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 96/31625</b> <b>(43) International Publication Date:</b> 10 October 1996 (10.10.96)
<b>(21) International Application Number:</b> PCT/US96/04454 <b>(22) International Filing Date:</b> 4 April 1996 (04.04.96) <b>(30) Priority Data:</b> 417,872                      7 April 1995 (07.04.95)                      US 630,915                      3 April 1996 (03.04.96)                      US <b>(71) Applicants:</b> CYTOGEN CORPORATION [US/US]; 600 College Road East, Princeton, NJ 08540 (US). UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL [US/US]; Office of Technology Development, CB#4100, 302 Bynum Hall, Chapel Hill, NC 27599-4105 (US). <b>(72) Inventors:</b> SPARKS, Andrew, B.; 201 Blue Ridge Road, Carrboro, NC 27510 (US). HOFFMAN, Noah; 5001 Manning Drive, Greensboro, NC 27410 (US). KAY, Brian, K.; 18 Wysteria Way, Chapel Hill, NC 27514 (US). FOWLKES, Dana, M.; 2013 Damascus Church Drive, Chapel Hill, NC 27516 (US). MCCONNELL, Stephen, J.; 10211 Camino Ruiz #52, San Diego, CA 92126 (US). <b>(74) Agents:</b> MISROCK, S., Leslie et al.; Pennie & Edmonds, 1155 Avenue of the Americas, New York, NY 10036 (US).		<b>(81) Designated States:</b> AL, AM, AU, AZ, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, IS, JP, KG, KP, KR, KZ, LK, LR, LS, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TM, TR, TT, UA, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> POLYPEPTIDES HAVING A FUNCTIONAL DOMAIN OF INTEREST AND METHODS OF IDENTIFYING AND USING SAME		
<b>(57) Abstract</b>  Novel polypeptides having functional domains of interest are described, along with DNA sequences that encode the same. A method of identifying these polypeptides by means of a sequence-independent (that is, independent of the primary sequence of the polypeptide sought), recognition unit-based functional screen is also disclosed. Various applications of the method and of the polypeptides identified are described, including their use in assay kits for drug discovery, modification, and refinement.		

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Latvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MR	Mauritania	UZ	Uzbekistan
GA	Gabon			VN	Viet Nam

**POLYPEPTIDES HAVING A FUNCTIONAL DOMAIN OF INTEREST  
AND METHODS OF IDENTIFYING AND USING SAME**

This application is a continuation-in-part of co-  
5 pending U.S. Patent Application Serial No. 08/417,872 filed  
April 7, 1995, the entire contents of which are incorporated  
herein by reference.

1. Introduction

10 The present invention is directed to polypeptides  
having a functional domain of interest or functional  
equivalents thereof. Methods of identifying these  
polypeptides are described, along with various methods of  
their use, including but not limited to targeted drug  
15 discovery.

2. Background of the Invention

Combinatorial libraries represent exciting new tools  
in basic science research and drug design. It is possible  
20 through synthetic chemistry or molecular biology to generate  
libraries of complex polymers, with many subunit permutations.  
There are many guises to these libraries: random peptides,  
which can be synthesized on plastic pins (Geysen et al., 1987,  
J. Immunol. Meth. 102:259-274), beads (Lam et al., 1991,  
25 Nature 354:82-84) or in a soluble form (Houghten et al., 1991,  
Nature 354:84-86) or expressed on the surface of viral  
particles (Cwirla et al., 1990, Proc. Natl. Acad. Sci. USA  
87:6378-6382; Kay et al., 1993, Gene 128:59-65; Scott and  
Smith, 1990, Science 249:386-390); nucleic acids (Ellington  
30 and Szostak, 1990, Nature 346:818-822; Gao et al., 1994, Proc.  
Natl. Acad. Sci. USA 91:11207-11211; Tuerk and Gold, 1990,  
Science 249:505-510); and small organic molecules (Gordon et  
al., 1994, J. Med. Chem. 37:1385-1401). These libraries are  
very useful in mapping protein-protein interactions and  
35 discovering drugs.

Phage display has become a powerful method for  
screening populations of peptides, mutagenized proteins, and

cDNAs for members that have affinity to target molecules of interest. It is possible to generate  $10^8$ - $10^9$  different recombinants from which one or more clones can be selected with affinity to antigens, antibodies, cell surface receptors, protein chaperones, DNA, metal ions, etc. Screening libraries is versatile because the displayed elements are expressed on the surface of the virus as capsid-fusion proteins. The most important consequence of this arrangement is that there is a physical linkage between phenotype and genotype. There are several other advantages as well: 1) virus particles which have been isolated from libraries by affinity selection can be regenerated by simple bacterial infection, and 2) the primary structure of the displayed binding peptide or protein can be easily deduced by DNA sequencing of the cloned segment in the viral genome.

Combinatorial peptide libraries have been expressed in bacteriophage. Synthetic oligonucleotides, fixed in length, but with multiple unspecified codons can be cloned into genes III, VI, or VIII of bacteriophage M13 where they are expressed as a plurality of peptide:capsid fusion proteins. The libraries, often referred to as random peptide libraries, can be screened for binding to target molecules of interest. Usually, three to four rounds of screening can be accomplished in a week's time, leading to the isolation of one to hundreds of binding phage.

The primary structure of the binding peptides is then deduced by nucleotide sequencing of individual clones. Inspection of the peptide sequences sometimes reveals a common motif, or consensus sequence. Generally, this motif when synthesized as a soluble peptide has the full binding activity. Random peptide libraries have successfully yielded peptides that bind to the Fab site of antibodies (Cwirla et al., 1990, Proc. Natl. Acad. Sci. USA 87:6378-6382; Scott and Smith, 1990, Science 249:386-390), cell surface receptors (Doorbar and Winter, 1994, J. Mol. Biol. 244:361-369; Goodson et al., 1994, Proc. Natl. Acad. Sci. USA 91:7129-7133), cytosolic receptors (Blond-Elguindi et al., 1993, Cell 75:717-

728), intracellular proteins (Daniels and Lane, 1994, J. Mol. Biol. 243:639-652; Dedman et al., 1993, J. Biol. Chem. 268:23025-23030; Sparks et al., 1994, J. Biol. Chem. 269:23853-23856), DNA (Krook et al., 1994, Biochem. Biophys. Res. Comm. 204:849-854), and many other targets (Winter, 1994, Drug Dev. Res. 33:71-89).

Most vital cellular processes are regulated by the transmission of signals throughout the cell in the form of complex interactions between proteins. As the study of signal transduction, or the flow of information throughout the cell, has broadened and matured, it has become apparent that these protein-protein interactions are often mediated by modular domains within signalling proteins. Src, both the first proto-oncogene product and the first tyrosine kinase discovered (Taylor and Shalloway, 1993, Current Opinion in Genetics and Development 3:26-34), is the prototypic modular domain-containing protein.

Src is a protein tyrosine kinase of 60 kilodaltons and is located at the plasma membrane of cells. It was first discovered in the 1970's to be the oncogenic element of Rous sarcoma virus, and in the 1980's, it was appreciated to be a component of the signal transduction system in animal cells. However, since the identification of viral and cellular forms of Src (i.e., v-Src and c-Src), their respective roles in oncogenesis, normal cell growth, and differentiation have not been completely understood.

In addition to its tyrosine kinase region (sometimes called a Src Homology 1 domain), Src contains two regions that have been found to have functionally and structurally homologous counterparts in a large number of proteins. These regions have been designated the Src Homology 2 (SH2) and Src Homology 3 (SH3) domains. SH2 and SH3 domains are modular in that they fold independently of the protein that contains them, their secondary structure places N-and C-termini close to one another in space, and they appear at variable locations (anywhere from N-to C-terminal) from one protein to the next (Cohen et al., 1995, Cell 80:237-248). SH2 domains have been

well-studied and are known to be involved in binding to phosphorylated tyrosine residues (Pawson and Gish, 1992, Cell 71:359-362).

The Src-homology region 3 (SH3) of Src is a domain 5 that is 60-70 amino acids in length and is present in many cellular proteins (Cohen et al., 1995, Cell 80:237-248; Pawson, 1995, Nature 373:573-580). Within Src, the SH3 domain is considered to be a negative inhibitory domain, because c-Src can be activated (i.e., transforming) through mutations in 10 this domain (Jackson et al., 1993, Oncogene 8:1943-1956; Seidel-Dugan et al., 1992, Mol Cell Biol 12:1835-1845).

To deduce the binding specificity of the Abl SH3 domain, a group led by David Baltimore screened cDNA libraries with radiolabeled GST-Abl SH3 fusion protein and identified 15 two binding cDNA clones (Cicchetti et al., 1992, Science 257:803-806). Both clones encoded proteins with proline rich regions that were later shown to be SH3 binding domains.

Subsequently, others have screened combinatorial peptide libraries and identified peptides that bound to the 20 Src SH3 domain (Yu et al., 1994, Cell 76:933-945; Cheadle et al., 1994, J. Biol. Chem. 269:24034-24039). Using the SH3 domain of Src, Sparks et al., 1994, J. Biol. Chem. 269:23853-23856 screened phage-display random peptide libraries and identified a consensus peptide sequence that binds with 25 specificity and high affinity to the Src SH3 domain.

The consensus from these various studies is that the optimal Src SH3 peptide ligand is RPLPPLP (SEQ ID NO:45). Recently, the structures of the peptide-SH3 domain complexes have been deduced by NMR and the peptides have been shown to 30 bind in two possible orientations with respect to the SH3 domain (Feng et al., 1994, Science 266:1241-1247; Lim et al., 1994, Nature 372:375-379).

Since SH3 domains have been found to have such important roles in the function of crucial signalling and 35 structural elements in the cell, a method of identifying proteins containing SH3 regions is of great interest. In this regard, it is important to note that such a method is

unavailable because of the low sequence similarity of modular functional domains, including SH3. See, e.g., Figure 6, which illustrates the minimal primary sequence homology among various known SH3 domains.

5           Sequence homology searches can potentially identify known proteins containing not yet recognized functional domains of interest, however, sequence homology generally needs to be >40% for this procedure to be successful. Functional domains generally are less than 40% homologous and  
10 therefore many would be missed in a sequence homology search. In addition, homology searches do not identify novel proteins; they only identify proteins already defined by nucleotide or amino acid sequence and present in the database.

          Another approach is to use hybridization techniques  
15 using nucleotide probes to search expression libraries for novel proteins. This method would have limited applicability to finding novel proteins containing functional domains due to the low sequence homology of the functional domains.

          Methods for isolating partner proteins involved in  
20 protein-protein interactions have generally focused on finding a ligand to a protein that has been found and characterized. Such approaches have included using anti-idiotypic antibodies that mimic the known protein to screen cDNA expression libraries for a binding ligand (Jerne, 1974, Ann. Immunol.  
25 (Inst. Pasteur) 125c:373-389; Sudol, 1994, Oncogene 9:2145-2152). Skolnick et al., 1991, Cell 65:83-90 isolated a binding partner for PI3-kinase by screening a cDNA expression library with the <sup>32</sup>P-labeled tyrosine phosphorylated carboxyl terminus of the epidermal growth factor receptor (EGFR).

30           An easy method for isolating operationally defined ligands involved in protein-protein interactions and for optimally identifying an exhaustive set of modular domain-containing proteins implicated in binding with the ligands would be highly desirable.

35           If such a method were available, however, such a method would be useful for the isolation of any polypeptide having a functioning version of any functional domain of

interest. Such a general method would be of tremendous utility in that whole families of related proteins each with its own version of the functional domain of interest could be identified. Knowledge of such related proteins would  
5 contribute greatly to our understanding of various physiological processes, including cell growth or death, malignancy, and immune reactions, to name a few. Such a method would also contribute to the development of increasingly more effective therapeutic, diagnostic, or  
10 prophylactic agents having fewer side effects.

According to the present invention, just such a method is provided.

Regarding SH3 domain-containing proteins, the method of the present invention will contribute greatly to our  
15 understanding of cell growth (Zhu et al., 1993, J. Biol. Chem. 268:1775-1779; Taylor and Shalloway, 1994, Nature 368:867-871), malignancy (Wages et al., 1992, J. Virol. 66:1866-1874; Bruton and Workman, 1993, Cancer Chemother. Pharmacol. 32:1-19), subcellular localization of proteins to the cytoskeleton  
20 and/or cellular membranes (Weng et al., 1993, J. Biol. Chem. 268:14956-14963; Bar-Sagi et al., 1993, Cell 74:83-91), signal transduction (Duchesne et al., 1993, Science 259:525-528), cell morphology (Wages et al., 1992, J. Virol. 66:1866-1874; McGlade et al., 1993, EMBO J. 12:3073-3081), neuronal  
25 differentiation Tanaka et al., 1993, Mol. Cell. Biol. 13:4409-4415), T cell activation (Reynolds et al., 1992, Oncogene 7:1949-1955), and cellular oxidase activity (McAdara and Babior, 1993, Blood 82:A28).

30 Citation of a reference hereinabove shall not be construed as an admission that such is prior art to the present invention.

### 3. SUMMARY OF THE INVENTION

35 In general, the present invention is directed to a method of using isolated, operationally defined ligands involved in binding interactions for optimally identifying an



exhaustive set of compounds binding to such ligands. In one embodiment, the isolated ligands are peptides involved in specific protein-protein interactions and are used to identify a set of novel modular domain-containing proteins that bind to the ligands. Using this method, proteins sharing only modest similarities but a common function can be found.

The present invention is directed to a method of identifying a polypeptide or family of polypeptides having a functional domain of interest. The basic steps of the method comprise: (a) choosing a recognition unit or set of recognition units having a selective affinity for a target molecule with a functional domain of interest; (b) contacting the recognition unit with a plurality of polypeptides; and (c) identifying a polypeptide having a selective binding affinity for the recognition unit, which polypeptide includes the functional domain of interest or a functional equivalent thereof.

In one particular embodiment of the invention, exhaustive screening of proteins having a desired functional domain involves an iterative process by which ligands or recognition units for SH3 domains identified in the first round of screening are used to detect SH3 domain-containing proteins in successive expression library screens.

More particularly, the method of the present invention includes choosing a recognition unit having a selective affinity for a target molecule with a functional domain of interest. With this recognition unit (particularly under the multivalent recognition unit screening conditions taught by the present invention), it has further been discovered that a plurality of polypeptides from various sources can be examined such that certain polypeptides having a selective binding affinity for the recognition unit can be identified. The polypeptides so identified have been shown to include the functional domain of interest; that is, the functional domains found are working versions that are capable of displaying the same binding specificity as the functional domain of interest. Hence, the polypeptides identified by the

present method also possess those attributes of the functional domain of interest which allow these related polypeptides to exhibit the same, similar, or analogous (but functionally equivalent) selective affinity characteristics as the domain of interest of the initial target molecule. By screening the plurality of peptides for recognition unit binding, the methods of the present invention circumvent the limitations of conventional DNA-based screening methods and allow for the identification of highly disparate protein sequences possessing functionally equivalent functional domains.

In specific embodiments of the present invention, the plurality of polypeptides is obtained from the proteins present in a cDNA expression library. The specificity of the polypeptides which bear the functional domain of interest or a functional equivalent thereof for various peptides or recognition units can subsequently be examined, allowing for a greater understanding of the physiological role of particular polypeptide/recognition unit interactions. Indeed, the present invention provides a method of targeted drug discovery based on the observed effects of a given drug candidate on the interaction between a recognition unit-polypeptide pair or a recognition unit and a "panel" of related polypeptides each with a copy or a functional equivalent of (e.g., capable of displaying the same binding specificity and thus binding to the same recognition unit as) the functional domain of interest.

The present invention also provides polypeptides comprising certain amino acid sequences. Moreover, the present invention also provides nucleic acids, including certain DNA constructs comprising certain coding sequences. Using the methods of the present invention, more than eighteen different SH3 domain-containing proteins have been identified, over half of which have not been previously described.

The present inventors have found, unexpectedly, that the valency (i.e., whether it is a monomer, dimer, tetramer, etc.) of the recognition unit that is used to screen an expression library or other source of polypeptides apparently

has a marked effect upon the specificity of the recognition unit-functional domain interaction. The present inventors have discovered that recognition units in the form of small peptides, in multivalent form, have a specificity that is eased but not forfeited. In particular, biotinylated peptides bound to a multivalent (believed to be tetravalent) streptavidin-alkaline phosphatase complex have an unexpected generic specificity. This allows such peptides to be used to screen libraries to identify classes of polypeptides containing functional domains that are similar but not identical in sequence to the peptides' original target functional domains.

The present invention also provides methods for identifying potential new drug candidates (and potential lead compounds) and determining the specificities thereof. For example, knowing that a polypeptide with a functional domain of interest and a recognition unit, e.g., a binding peptide, exhibit a selective affinity for each other, one may attempt to identify a drug that can exert an effect on the polypeptide-recognition unit interaction, e.g., either as an agonist or as an antagonist (inhibitor) of the interaction. With this assay, then, one can screen a collection of candidate "drugs" for the one exhibiting the most desired characteristic, e.g., the most efficacious in disrupting the interaction or in competing with the recognition unit for binding to the polypeptide.

In addition, the present invention also provides certain assay kits and methods of using these assay kits for screening drug candidates for their ability to affect the binding of a polypeptide containing a functional domain to a recognition unit. In a particular aspect of the present invention, the assay kit comprises: (a) a polypeptide containing a functional domain of interest; and (b) a recognition unit having a selective binding affinity for the polypeptide. Yet another assay kit may comprise a plurality of polypeptides, each polypeptide containing a functional domain of interest, in which the functional domain of interest

is a domain selected from the group consisting of an SH1, SH2, SH3, PH, PTB, LIM, armadillo, Notch/ankyrin repeat, zinc finger, leucine zipper, and helix-turn-helix, and at least one recognition unit having a selective affinity for each of the  
5 plurality of polypeptides.

Other objects of the present invention will be apparent to those of ordinary skill upon further consideration of the following detailed description.

#### 10 4. DESCRIPTION OF THE FIGURES

Figure 1 is a schematic representation of the general aspects of a method of identifying recognition units exhibiting a selective affinity for a target molecule with a functional domain of interest. In this illustration, the  
15 target molecule is a polypeptide with an SH3 domain, and the recognition units are peptides having a selective affinity for the SH3 domain that are expressed in a phage displayed library.

20 Figure 2 illustrates the selectivities exhibited by particular recognition units that bind to the Src SH3 domain (in this case, two heptapeptides) for a "panel" of known polypeptides known to contain an SH3 domain. The non-SH3-containing protein, GST, serves as control. RPLPPLP is (SEQ  
25 ID NO:45); APPVPPR is (SEQ ID NO:203)

Figure 3 is a schematic representation of the general method of identifying polypeptides with a functional domain of interest by screening a plurality of polypeptides  
30 using a suitable recognition unit. In the illustration, the plurality of polypeptides is obtained from a cDNA expression library, and the recognition units are SH3 domain-binding peptides.

35 Figure 4 illustrates how an SH3 domain-binding peptide can be used to identify other SH3 domain-containing proteins. Shown is a schematic representation of the

progression from initial selection of a target molecule with a functional domain of interest, choice of recognition unit, and identification of polypeptides that have a selective affinity for the recognition unit and include the functional domain of interest or a functional equivalent thereof.

Figure 5 depicts filters from primary (Figure 5B) and tertiary (Figure 5A) screens of a  $\lambda$ cDNA library probed with a biotinylated SH3-binding peptide recognition unit in the form of a complex with streptavidin-alkaline phosphatase (SA-AP). A mouse 16 day embryo cDNA library in  $\lambda$ EXlox was incubated with a multivalent complex formed between biotinylated pSrcCII and SA-AP. The sites of peptide binding were detected by incubation with BCIP (5-bromo-4-chloro-3-indoyl-phosphate-p-toluidine salt) and NBT (nitroblue tetrazolium chloride) for approximately five minutes.

Figure 6 shows an alignment of SH3 domains that illustrates the minimal primary sequence homology among various known SH3 domains. The amino acid sequences shown are SEQ ID NOS:68-111.

Figure 7A is a schematic representation of a population of functional domains represented by the circles. "A" is a recognition unit specific to one circle only. B, on the other hand, recognizes three domains, while B1 and B2 recognize only two each. Figure 7B illustrates an iterative method whereby new recognition units are chosen based on polypeptides uncovered with the first recognition unit(s). These new recognition units lead to the identification of other related polypeptides, etc., expanding the scope of the study to increasingly diverse members of the related population.

Figure 8 illustrates the binding specificity of several SH3 domain recognition units. Biotinylated Class I (pSrcCI) or Class II (pSrcCII) Src SH3 domain recognition

units, Crk SH3 domain recognition units (pCrk), PLC $\gamma$  SH3 domain recognition units (pPLC), and Abl SH3 domain recognition units (pAbl) were tested for binding to the indicated GST-SH3 domain fusion proteins immobilized onto  
5 duplicate microtiter plate wells. Recognition units are listed along the left side of the figure; GST-SH3 domain fusion proteins are listed along the bottom. Recognition units were incubated either as multivalent complexes of biotinylated peptides and streptavidin-horseradish peroxidase  
10 (SA-HRP) (complexed) or as monovalent biotinylated peptides (uncomplexed), followed by incubation with SA-HRP. Average optical densities are shown.

Figure 9 shows a schematic of SH3-domain containing  
15 proteins isolated using the present invention. The name, identity, type of screen, and number of individual clones derived for each sequence are indicated. Diagrams are to scale, with SH3 domains representing approximately 60 amino acids. The abbreviations AR, P, CR, E/P, and SH2 represent  
20 ankyrin repeats, proline-rich segments, Cortactin repeats, glutamate/proline-rich segments, and Src homology 2 domains, respectively. Flared ends represent putative translation initiation sites for individual cDNAs. The Mouse, Human 1, and Human 2 libraries correspond to mouse 16 day embryo, human  
25 bone marrow, and human prostate cancer cDNA libraries, respectively. For a description of the pSrcII and pCort recognition units, see Section 6.1.

Figure 10A and 10B depicts the sequence alignment of  
30 SH3 domains in proteins isolated using the present invention. The name and identity of each clone is indicated. Where appropriate, multiple SH3 domains from the same polypeptide are designated A, B, C, etc., from N- to C-terminal. Periods indicate gaps introduced to maximize alignment of similar  
35 residues. Positions corresponding to conserved residues shown to be involved in ligand binding in the SH3 domains of Src and Grb2/Sem5 (Tomasetto et al., 1995, Genomics 28:367-376) are

presented in bold and underlined, respectively. Primary structures of SH3P1-8 and SH3P10-13 correspond to mouse, SH3P15-18, clone 5, 34, 40, 41, 45, 53, 55, 56, and 65 to human, and SH3P9 and SH3P14 to mouse (m) or human (h) cDNA clones. For sequence comparison, the sequence of the mouse c-Src SH3 domain (GenBank accession number P41240) is shown. The GenBank accession numbers for mouse Cortactin, SPY75/HS1, Crk, and human MLN50, Lyn, Fyn, and Src are U03184, D42120, S72408, X82456, M16038, P06241, and P41240, respectively. The amino acid sequences shown are SEQ ID NOS:112-140.

Figure 11 depicts the specificity continuum described in Section 5.2.1. "SA-AP peptide complex" represents the multivalent (believed to be tetravalent) complex of streptavidin-alkaline phosphatase and biotinylated peptide described in that section.

Figure 12 depicts the results of experiments in which peptide recognition units were synthesized and tested for their ability to bind to novel SH3 domains described in Sections 6.1 and 6.1.1. A minus indicates no binding; a plus indicates binding, with the number of pluses indicating the strength of binding. For further details, see Section 6.2. The amino acid sequences shown are SEQ ID NOS:141-168.

25

Figure 13 depicts more data from the experiment depicted in Figure 12. The amino acid sequences shown are SEQ ID NOS:169-188.

30

Figure 14 illustrates the effect of preconjugation with streptavidin-alkaline phosphatase on the affinity of biotinylated peptides for SH3 domains. See Section 6.3.1 for details.

35

Figure 15 illustrates the effect of preconjugation with streptavidin-alkaline phosphatase on the specificity of biotinylated peptides for GST-SH3 domain fusion proteins that

have been immobilized on nylon membranes. See Section 6.3.2 for details.

Figure 16 illustrates the effect of preconjugation with streptavidin-alkaline phosphatase on the specificity of biotinylated peptides for proteins containing SH3 domains expressed by cDNA clones. See Section 6.3.3 for details.

Figure 17 illustrates a strategy for exhaustively screening an expression library for SH3 domain-containing proteins. A peptide recognition unit is generated by screening a combinatorial peptide library for binders to an SH3 domain expressed bacterially as a GST fusion protein. This peptide is then used as a multivalent streptavidin-biotinylated peptide complex to screen for a subset of the SH3 domain-containing proteins represented in a cDNA expression library. A combinatorial library is once again used to identify recognition units of SH3 domains identified in the first expression library screen; these recognition units identify overlapping sets of proteins from the expression library. With multiple iterations of this process, it should be possible to clone systematically all SH3 domains represented in a given cDNA expression library.

Figure 18 depicts the nucleotide sequence of SH3P1, mouse p53bp2 (SEQ ID NO:5).

Figure 19 depicts the amino acid sequence of SH3P1, mouse p53bp2 (SEQ ID NO:6).

Figure 20 depicts the nucleotide sequence of SH3P2, a novel mouse gene (SEQ ID NO:7).

Figure 21 depicts the amino acid sequence of SH3P2, a novel mouse gene (SEQ ID NO:8).



Figure 22 depicts the nucleotide sequence of SH3P3,  
a novel mouse gene (SEQ ID NO:9).

Figure 23 depicts the amino acid sequence of SH3P3,  
5 a novel mouse gene (SEQ ID NO:10).

Figure 24 depicts the nucleotide sequence of SH3P4,  
a novel mouse gene (SEQ ID NO:11).

10 Figure 25 depicts the amino acid sequence of SH3P4,  
a novel mouse gene (SEQ ID NO:12).

Figure 26 depicts the nucleotide sequence of SH3P5,  
mouse Cortactin (SEQ ID NO:13).  
15

Figure 27 depicts the amino acid sequence of SH3P5,  
mouse Cortactin (SEQ ID NO:14).

Figure 28 depicts the nucleotide sequence of SH3P6,  
20 mouse MLN50 (SEQ ID NO:15).

Figure 29 depicts the amino acid sequence of SH3P6,  
mouse MLN50 (SEQ ID NO:16).

25 Figure 30 depicts the nucleotide sequence of SH3P7,  
a novel mouse gene (SEQ ID NO:17).

Figure 31 depicts the amino acid sequence of SH3P7,  
a novel mouse gene (SEQ ID NO:18).  
30

Figure 32 depicts the nucleotide sequence of SH3P8,  
a novel mouse gene (SEQ ID NO:19).

Figure 33 depicts the amino acid sequence of SH3P8,  
35 a novel mouse gene (SEQ ID NO:20)..

Figure 34 depicts the nucleotide sequence of SH3P9, a novel mouse gene (SEQ ID NO:21).

Figure 35 depicts the amino acid sequence of SH3P9, a novel mouse gene (SEQ ID NO:22).

Figure 36 depicts the nucleotide sequence of SH3P9, a novel human gene (SEQ ID NO:23).

10 Figure 37 depicts the amino acid sequence of SH3P9, a novel human gene (SEQ ID NO:24).

Figure 38 depicts the nucleotide sequence of SH3P10, mouse HS1 (SEQ ID NO:25).

15 Figure 39 depicts the amino acid sequence of SH3P10, mouse HS1 (SEQ ID NO:26).

Figure 40 depicts the nucleotide sequence of SH3P11, 20 mouse Crk (SEQ ID NO:27).

Figure 41 depicts the amino acid sequence of SH3P11, mouse Crk (SEQ ID NO:28).

25 Figure 42A depicts the nucleotide sequence from positions 1-2600 of SH3P12, a novel mouse gene (a portion of SEQ ID NO:29).

Figure 42B depicts the nucleotide sequence from 30 positions 2601-3335 of SH3P12, a novel mouse gene (a portion of SEQ ID NO:29).

Figure 43 depicts the amino acid sequence of SH3P12, a novel mouse gene (SEQ ID NO:30).

35 Figure 44 depicts the nucleotide sequence of SH3P13, a novel mouse gene (SEQ ID NO:31).

Figure 45 depicts the amino acid sequence of SH3P13, a novel mouse gene (SEQ ID NO:32).

Figure 46A depicts the nucleotide sequence from 5 positions 1-2400 of SH3P14, mouse H74 (a portion of SEQ ID NO:33).

Figure 46B depicts the nucleotide sequence from positions 2351-4091 of SH3P14, mouse H74 (a portion of SEQ ID NO:33).

Figure 47 depicts the amino acid sequence of SH3P14, mouse H74 (SEQ ID NO:34).

15 Figure 48 depicts the nucleotide sequence of SH3P14, human H74 (SEQ ID NO:35).

Figure 49 depicts the amino acid sequence of SH3P14, human H74 (SEQ ID NO:36).

20

Figure 50 depicts the nucleotide sequence of SH3P17, a novel human gene (SEQ ID NO:37).

Figure 51 depicts the amino acid sequence of SH3P17, 25 a novel human gene (SEQ ID NO:38).

Figure 52A depicts the nucleotide sequence of SH3P18, a novel human gene (SEQ ID NO:39).

30 Figure 53 depicts the amino acid sequence of SH3P18, a novel human gene (SEQ ID NO:40).

Figure 54 depicts the nucleotide sequence of clone 55, a novel human gene (SEQ ID NO:189).

35

Figure 55 depicts the amino acid sequence of clone 55, a novel human gene (SEQ ID NO:190).

Figure 56 depicts the nucleotide sequence of clone 56, a novel human gene (SEQ ID NO:191).

Figure 57 depicts the amino acid sequence of clone 56, a novel human gene (SEQ ID NO:192).

Figure 58A depicts the nucleotide sequence from position 1-1720 of clone 65, a novel human gene (a portion of SEQ ID NO:193).

10

Figure 58B depicts the nucleotide sequence from position 1721-2873 of clone 65, a novel human gene (a portion of SEQ ID NO:193).

15

Figure 59 depicts the amino acid sequence of clone 65, a novel human gene (SEQ ID NO:194).

Figure 60 depicts the nucleotide sequence of clone 34, a novel human gene (SEQ ID NO:195).

20

Figure 61A depicts a portion of the amino acid sequence of clone 34, a novel human gene (a portion of SEQ ID NO:196).

25

Figure 61B depicts a portion of the amino acid sequence of clone 34, a novel human gene (a portion of SEQ ID NO:196).

Figure 62 depicts the nucleotide sequence of clone 41, a novel human gene (SEQ ID NO:197).

30

Figure 63A depicts a portion of the amino acid sequence of clone 41, a novel human gene (a portion of SEQ ID NO:198).

35

Figure 63B depicts a portion of the amino acid sequence of clone 41, a novel human gene (a portion of SEQ ID NO:198).

5           Figure 64A depicts the nucleotide sequence of clone 53, a novel human gene (SEQ ID NO:199).

Figure 65A depicts a portion of the amino acid sequence of clone 53, a novel human gene (a portion of SEQ ID  
10 NO:200).

Figure 65B depicts a portion of the amino acid sequence of clone 53, a novel human gene (a portion of SEQ ID NO:200).

15

Figure 66A and 66B depicts the nucleotide sequence (SEQ ID NO:220) and amino acid sequence (SEQ ID NO:221) of clone 5, a novel human gene.

20 5.   DETAILED DESCRIPTION OF THE INVENTION

As stated above, the present invention is related broadly to certain polypeptides having a functional domain of interest and is directed to methods of identifying and using these polypeptides. The present invention is also directed to  
25 a method of using isolated, operationally defined ligands involved in binding interactions for optimally identifying an exhaustive set of compounds binding such ligands and to compounds, target molecules, and, in one embodiment, polypeptides having a functional domain of interest and to  
30 methods of using these compounds. The detailed description that follows is provided to elucidate the invention further and to assist further those of ordinary skill who may be interested in practicing particular aspects of the invention.

First, certain definitions are in order.

35 Accordingly, the term "polypeptide" refers to a molecule comprised of amino acid residues joined by peptide (i.e., amide) bonds and includes proteins and peptides. Hence, the

polypeptides of the present invention may have single or multiple chains of covalently linked amino acids and may further contain intrachain or interchain linkages comprised of disulfide bonds. Some polypeptides may also form a subunit of 5 a multiunit macromolecular complex. Naturally, the polypeptides can be expected to possess conformational preferences and to exhibit a three-dimensional structure. Both the conformational preferences and the three-dimensional structure will usually be defined by the polypeptide's primary 10 (i.e., amino acid) sequence and/or the presence (or absence) of disulfide bonds or other covalent or non-covalent intrachain or interchain interactions.

The polypeptides of the present invention can be any size. As can be expected, the polypeptides can exhibit a wide 15 variety of molecular weights, some exceeding 150 to 200 kilodaltons (kD). Typically, the polypeptides may have a molecular weight ranging from about 5,000 to about 100,000 daltons. Still others may fall in a narrower range, for example, about 10,000 to about 75,000 daltons, or about 20,000 20 to about 50,000 daltons.

The phrase "functional domain" refers to a region of a polypeptide which affords the capacity to perform a particular function of interest. This function may give rise to a biological, chemical, or physiological consequence that 25 may be reversible or irreversible and which may include, but not be limited to, protein-protein interactions (e.g., binding interactions) involving the functional domain, a change in the conformation or a transformation into a different chemical state of the functional domain or of molecules acted upon by 30 the functional domain, the transduction of an intracellular or intercellular signal, the regulation of gene or protein expression, the regulation of cell growth or death, or the activation or inhibition of an immune response. Furthermore, the functional domain of interest is defined by a particular 35 functional domain that is present in a given target molecule. A discussion of the selection of a particular functional domain-containing target molecule is presented further below.

Many functional domains tend to be modular in that such domains may occur one or more times in a given polypeptide (or target molecule) or may be found in a family of different polypeptides. When found more than once in a  
5 given polypeptide or in different polypeptides, the modular functional domain may possess substantially the same structure, in terms of primary sequence and/or three-dimensional space, or may contain slight or great variations or modifications among the different versions of the  
10 functional domain of interest.

What is important, however, is that these related functional domains retain the functional aspects of the functional domain of interest present in the target molecule. It is stressed that, indeed, it is this functional  
15 relationship among two or more possible versions of a functional domain of interest which may be identified, defined, and exploited by the methods of the present invention. In a preferred aspect, the function of interest is the ability to bind to a molecule (e.g., a peptide) of  
20 interest.

The present invention provides a general strategy by which recognition units that bind to a functional domain-containing molecule can be used to screen expression libraries of genes (e.g., cDNA, genomic libraries) systematically for  
25 novel functional domain-containing proteins. In specific embodiments, the recognition units are prior isolated from a random peptide library, or are known peptide ligands or recognition units, or are recognition units that are identified by database searches for sequences having homology  
30 to a peptide recognition unit having the binding specificity of interest. Using the methods of the present invention, it is possible to exhaustively screen an expression library for proteins with a given functional domain.

In the prior art, novel genes (and thus their  
35 encoded protein products) are most commonly identified from cDNA libraries. Generally, an appropriate cDNA library is screened with a probe that is either an oligonucleotide or an

antibody. In either case, the probe must be specific enough for the gene that is to be identified to pick that gene out from a vast background of non-relevant genes in the library. It is this need for a specific probe that is the highest  
5 hurdle that must be overcome in the prior art identification of novel genes. Another method of identifying genes from cDNA libraries is through use of the polymerase chain reaction (PCR) to amplify a segment of a desired gene from the library. PCR requires that oligonucleotides having sequence similarity  
10 to the desired gene be available.

If the probe used in prior art methods is a nucleic acid, the cDNA library may be screened without the need for expressing any protein products that might be encoded by the cDNA clones. If the probe used in prior art methods is an  
15 antibody, then it is necessary to build the cDNA library into a suitable expression vector. For a comprehensive discussion of the art of identifying genes from cDNA libraries, see Sambrook, Fritsch, and Maniatis, "Construction and Analysis of cDNA Libraries," Chapter 8 in Cloning, A Laboratory Manual, 2d  
20 ed., Cold Spring Harbor Laboratory Press, 1989. See also Sambrook, Fritsch, and Maniatis, "Screening Expression Libraries with Antibodies and Oligonucleotides," Chapter 12 in Cloning, A Laboratory Manual, 2d ed., Cold Spring Harbor Laboratory Press, 1989.

25 As an alternative to cDNA libraries, genomic libraries are used. When genomic libraries are used in prior art methods, the probe is virtually always a nucleic acid probe. See Sambrook, Fritsch, and Maniatis, "Analysis and Cloning of Eukaryotic Genomic DNA," Chapter 9 in Cloning, A  
30 Laboratory Manual, 2d ed., Cold Spring Harbor Laboratory Press, 1989.

In the prior art, nucleic acid probes used in screening libraries are often based upon the sequence of a known gene that is thought to be homologous to a gene that it  
35 is desired to isolate. The success of the procedure depends upon the degree of homology between the probe and the target gene being sufficiently high. Probes based upon the sequences



of known functional domains in proteins had limited value because, while the sequences of the functional domains were similar enough to allow for their recognition as shared domains, the similarity was not so high that the probes could  
5 be used to screen cDNA or genomic libraries for genes containing the functional domains.

PCR may also be used to identify genes from genomic libraries. However, as in the case of using PCR to identify genes from cDNA libraries, this requires that oligonucleotides  
10 having sequence similarity to the desired gene be available.

Using the screening methods provided by the present invention, DNA encoding proteins having a desired functional domain that would not be readily identified by sequence homology can be identified by functional binding specificity  
15 to recognition units. By virtue of an ease in specificity of binding requirements conferred by the screening methods of the present invention, many novel, functionally homologous, functional domain-containing proteins can be identified. Although not intending to be bound by any mechanistic  
20 explanation, this ease in binding specificity is believed to be the result of the use of a multivalent peptide recognition unit used to screen the gene library, preferably of a valency greater than bivalent, more preferably tetravalent or greater, and most preferably the streptavidin-biotinylated peptide  
25 recognition unit complex.

In one particular embodiment of the invention, exhaustive screening of proteins having a desired functional domain involves an iterative process by which recognition units for SH3 domains identified in the first round of  
30 screening are used to detect SH3 domain-containing proteins in successive expression library screens (see Figure 17). This strategy enables one to search "sequence space" in what might be thought of as ever-widening circles with each successive cycle. This iterative strategy can be initiated even when  
35 only one functional domain-containing protein and recognition unit are available.

This iterative process is not limited to proteins containing SH3 domains. Members within a class of other functional domains also tend to have overlapping, or at least similar recognition unit preferences, are structurally stable, and often confer similar binding properties to a wide variety of proteins. These characteristics predict that the methods of the present invention will be applicable to a wide variety of functional domain-containing proteins in addition to their applicability to SH3 domain-containing proteins.

10

#### 5.1. Discovery of Novel Genes and Polypeptides Containing Functional Domains

The present invention provides methods for the identification of one or more polypeptides (in particular, a "family" of polypeptides, including the target molecule) that contains a functional domain of interest that either corresponds to or is the functional equivalent of a functional domain of interest present in a predetermined target molecule.

The present invention provides a mechanism for the rapid identification of genes (e.g., cDNAs) encoding virtually any functional domain of interest. By screening cDNA libraries or other sources of polypeptides for recognition unit binding rather than sequence similarity, the present invention circumvents the limitations of conventional DNA-based screening methods and allows for the identification of highly disparate protein sequences possessing equivalent functional activities. The ability to isolate entire repertoires of proteins containing particular modular functional domains will prove invaluable both in molecular biological investigations of the genome and in bringing new targets into drug discovery programs.

It should likewise be apparent that a wide range of polypeptides having a functional domain of interest can be identified by the process of the invention, which process comprises:

(a) contacting a multivalent recognition unit complex with a plurality of polypeptides; and

(b) identifying a polypeptide having a selective binding affinity for said recognition unit complex.

In a specific embodiment, the process comprises:

(a) contacting a multivalent recognition unit  
5 complex with a plurality of polypeptides from which it is desired to identify a polypeptide having selective binding affinity for the recognition unit, in which the valency of the recognition unit in the complex is at least two, or at least four; and

10 (b) identifying, and preferably recovering, a polypeptide having a selective binding affinity for the recognition unit complex.

In another specific embodiment, the process comprises a method of identifying at least one polypeptide  
15 comprising a functional domain of interest, said method comprising:

(a) contacting one or more multivalent recognition unit complexes with a plurality of polypeptides; and

(b) identifying at least one polypeptide having  
20 selective binding affinity for at least one of said recognition unit complexes.

In another specific embodiment, the process comprises:

(a) contacting a multivalent recognition unit  
25 complex, which complex comprises (i) avidin or streptavidin, and (ii) biotinylated recognition units, with a plurality of polypeptides from a cDNA expression library, in which the recognition unit is a peptide having in the range of 6 to 60 amino acid residues; and

30 (b) identifying a polypeptide having a selective binding affinity for said recognition unit complex.

In another specific embodiment, the process comprises a method of identifying a polypeptide having an SH3 domain of interest comprising:

35 (a) contacting a multivalent recognition unit complex, which complex comprises (i) avidin or streptavidin, and (ii) biotinylated recognition units, with a plurality of

polypeptides from a cDNA expression library, in which the recognition unit is a peptide having in the range of 6 to 60 amino acid residues and which selectively binds an SH3 domain; and

- 5 (b) identifying a polypeptide having a selective binding affinity for said recognition unit complex.

In another specific embodiment, the process comprises a method of identifying a polypeptide having a functional domain of interest or a functional equivalent  
10 thereof comprising:

(a) screening a random peptide library to identify a peptide that selectively binds a functional domain of interest; and

(b) screening a cDNA or genomic expression library  
15 with said peptide or a binding portion thereof to identify a polypeptide that selectively binds said peptide.

In a specific embodiment of the above method, the screening step (b) is carried out by use of said peptide in the form of multiple antigen peptides (MAP) or by use of said  
20 peptide cross-linked to bovine serum albumin or keyhole limpet hemocyanin.

In another specific embodiment, the process comprises a method of identifying a polypeptide having a functional domain of interest or a functional equivalent  
25 thereof comprising:

(a) screening a random peptide library to identify a plurality of peptides that selectively bind a functional domain of interest;

(b) determining at least part of the amino acid  
30 sequences of said peptides;

(c) determining a consensus sequence based upon the determined amino acid sequences of said peptides; and

(d) screening a cDNA or genomic expression library with a peptide comprising the consensus sequence to identify a  
35 polypeptide that selectively binds said peptide.

In another specific embodiment, the process comprises a method of identifying a polypeptide having a

functional domain of interest or a functional equivalent thereof comprising:

(a) screening a random peptide library to identify a first peptide that selectively binds a functional domain of interest;

(b) determining at least part of the amino acid sequence of said first peptide;

(c) searching a database containing the amino acid sequences of a plurality of expressed natural proteins to identify a protein containing an amino acid sequence homologous to the amino acid sequence of said first peptide; and

(d) screening a cDNA or genomic expression library with a second peptide comprising the sequence of said protein that is homologous to the amino acid sequence of said first peptide.

The identified polypeptide identified by the above-described methods thus should contain the functional domain of interest or a functional equivalent thereof (that is, having a functional domain that is identical, or having a functional domain that differs in sequence but is capable of binding to the same recognition unit). In a particular embodiment, the polypeptide identified is a novel polypeptide. In a preferred embodiment, the recognition unit that is used to form the multivalent recognition unit complex is isolated or identified from a random peptide library.

In a specific embodiment, the present invention provides amino acid sequences and DNA sequences encoding novel proteins containing SH3 domains. The SH3 domains vary in sequence but retain binding specificity to an SH3 domain recognition unit. Also provided are fragments and derivatives of the novel proteins containing SH3 domains as well as DNA sequences encoding the same. It will be apparent to one of ordinary skill in the art that also provided are proteins that vary slightly in sequence from the novel proteins by virtue of conservative amino acid substitutions. It will also be apparent to one of ordinary skill in the art that the novel

proteins may be expressed recombinantly by standard methods. The novel proteins may also be expressed as fusion proteins with a variety of other proteins, e.g., glutathione S-transferase.

5           The present invention provides a purified polypeptide comprising an SH3 domain, said SH3 domain having an amino acid sequence selected from the group consisting of: SEQ ID NOs: 113-115, 118-121, 125-128, 133-139, 204-218, and 219. Also provided is a purified DNA encoding the  
10 polypeptide.

          Also provided is a purified polypeptide comprising an SH3 domain, said polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 10, 12, 18, 20, 22, 24, 30, 32, 38, 40, 190, 192, 194, 196, 198, 200,  
15 and 221. Also provided is a purified DNA encoding the polypeptide.

          Also provided is a purified DNA encoding an SH3 domain, said DNA having a sequence selected from the group consisting of SEQ ID NOs: 7, 9, 11, 17, 19, 21, 23, 29, 31,  
20 37, 39, 189, 191, 193, 195, 197, 199, and 220. Also provided is a nucleic acid vector comprising this purified DNA. Also provided is a recombinant cell containing this nucleic acid vector.

          Also provided is a purified DNA encoding a  
25 polypeptide having an amino acid sequence selected from the group consisting of: SEQ ID NOs: 8, 10, 12, 18, 20, 22, 24, 30, 32, 38, 40, 190, 192, 194, 196, 198, 200, and 221. Also provided is a nucleic acid vector comprising this purified DNA. Also provided is a recombinant cell containing this  
30 nucleic acid vector.

          Also provided is a purified DNA encoding a polypeptide comprising an amino acid sequence selected from the group consisting of: SEQ ID NOs: 113-115, 118-121, 125-128, 133-139, 204-218, and 219. Also provided is a nucleic acid  
35 vector comprising this purified DNA. Also provided is a recombinant cell containing this nucleic acid vector.

Also provided is a purified molecule comprising an SH3 domain of a polypeptide having an amino acid sequence selected from the group consisting of: SEQ ID NO: 8, 10, 12, 18, 20, 22, 24, 30, 32, 38, 40, 190, 192, 194, 196, 198, 200, 5 and 221.

Also provided is a fusion protein comprising (a) an amino acid sequence comprising an SH3 domain of a polypeptide having the amino acid sequence of SEQ ID NO: 8, 10, 12, 18, 20, 22, 24, 30, 32, 38, 40, 190, 192, 194, 196, 198, 200, and 10 221 joined via a peptide bond to (b) an amino acid sequence of at least six, or ten, or twenty amino acids from a different polypeptide. Also provided is a purified DNA encoding the fusion protein. Also provided is a nucleic acid vector comprising the purified DNA encoding the fusion protein. Also 15 provided is a recombinant cell containing this nucleic acid vector. Also provided is a method of producing this fusion protein comprising culturing a recombinant cell containing a nucleic acid vector encoding said fusion protein such that said fusion protein is expressed, and recovering the expressed 20 fusion protein.

The present invention also provides a purified nucleic acid hybridizable to a nucleic acid having a sequence selected from the group consisting of: SEQ ID NOS: 7, 9, 11, 17, 19, 21, 23, 29, 31, 37, 39, 189, 191, 193, 195, 197, 199, 25 and 220.

The present invention also provides antibodies to a polypeptide having an amino acid sequence selected from the group consisting of: SEQ ID NOS: 113-115, 118-121, 125-128, 133-139, 204-218, and 219.

30 The present invention also provides antibodies to a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NOS: 8, 10, 12, 18, 20, 22, 24, 30, 32, 38, 40, 190, 192, 194, 196, 198, 200, and 221.

It is demonstrated by way of example herein that 35 recognition units that comprise SH3 domain ligands derived from combinatorial peptide libraries may be used in the methods of the present invention as probes for the rapid

discovery of novel proteins containing SH3 functional domains. The methods of the present invention require no prior knowledge of the characteristics of a SH3 domain's natural cellular ligand to initiate the process of discovery. One  
5 needs only enough purified SH3 domain-containing protein (by way of example, 1-5 $\mu$ g) to select peptides from a random peptide library. In addition, because the methods of the present invention identify novel proteins from cDNA expression libraries based only on their binding properties, low primary  
10 sequence identity between the target SH3 domain and the SH3 domains of the novel proteins discovered need not be a limitation, provided some functional similarity between these SH3 domains is conserved. Also, the methods of the present invention are rapid, require inexpensive reagents, and employ  
15 simple and well established laboratory techniques.

Using these methods, more than eighteen different SH3 domain-containing proteins have been identified, over half of which have not been previously described. While certain of these previously unknown proteins are clearly related to known  
20 genes such as amphiphysin and drebrin, others constitute new classes of signal transduction and/or cytoskeletal proteins. These include SH3P17 and SH3P18, two members of a new family of adaptor-like proteins comprised of multiple SH3 domains; SH3P12, a novel protein with three SH3 domains and a region  
25 similar to the extracellular peptide hormone sorbin; and SH3P4, SH3P8, and SH3P13, three members of a third new family of SH3-containing proteins. These novel proteins are described more fully in Sections 6.1 and 6.1.1. The high incidence of novel proteins identified by the methods of the  
30 present invention indicates that a large number of SH3 domain-containing proteins remain to be discovered by application of the methods of the invention.

One of ordinary skill in the art would recognize that the above-described novel proteins need not be used in  
35 their entirety in the various applications of those proteins described herein. In many cases it will be sufficient to employ that portion of the novel protein that contains the



functional (e.g., SH3) domain. Such exemplary portions of SH3 domain-containing proteins are shown in Figure 10A and 10B. Accordingly, the present invention provides derivatives (e.g., fragments and molecules comprising these fragments) of novel proteins that contain SH3 domains, e.g., as shown in Figure 10A and 10B. Nucleic acids encoding these fragments or other derivatives are also provided.

In another embodiment, the present invention includes a method of identifying one or more novel polypeptides having an SH3 domain, said method comprising:

- (a) identifying a recognition unit having a selective affinity for the SH3 domain by screening a peptide library with the SH3 domain;
- (b) producing said recognition unit;
- (c) contacting said recognition unit with a source of polypeptides; and
- (d) identifying one or more novel polypeptides having a selective affinity for said recognition unit, which polypeptides comprise the SH3 domain.

#### 5.1.1 Functional Domains

Functional domains of interest in the practice of the present invention can take many forms and may perform a variety of functions. For example, such functional domains may be involved in a number of cellular, biochemical, or physiological processes, such as cellular signal transduction, transcriptional regulation, translational regulation, cell adhesion, migration or transport, cytokine secretion and other aspects of the immune response, and the like. In particular embodiments of the present invention, the functional domains of interest may consist of regions known as SH1, SH2, SH3, PH, PTB, LIM, armadillo, and Notch/ankyrin repeat. See, e.g., Pawson, 1995, Nature 373:573-580; Cohen et al., 1995, Cell 80:237-248. Functional domains may also be chosen from among regions known as zinc fingers, leucine zippers, and helix-turn-helix or helix-loop-helix. Certain functional domains may be binding domains, such as DNA-binding domains or actin-

binding domains. Still other functional domains may serve as sites of catalytic activity.

In one embodiment of the invention, a suitable target molecule containing the chosen functional domain of interest is selected. In the case of an SH3 domain, for example, a number of proteins (or functional domain-containing derivatives or analogs thereof) may be selected as the target molecule, including but not limited to, the Src family of proteins: Fyn, Lck, Lyn, Src, or Yes. Still other proteins contain an SH3 domain and can be used, including, but not limited to: Abl, Crk, Nck (other oncogenes), Grb2, PLC $\gamma$ , RasGAP (proteins involved in signal transduction), ABP-1, myosin-1, spectrin (proteins found in the cytoskeleton), and neutrophil NADPH oxidase (an enzyme). In the case of a catalytic site, any catalytically active protein, such as an enzyme, can be used, particularly one whose catalytic site is known. For example, the catalytic site of the protein glutathione S-transferase (GST) can be used. Other target molecules that possess catalytic activity may include, but are not limited to, protein serine/threonine kinases, protein tyrosine kinases, serine proteases, DNA or RNA polymerases, phospholipases, GTPases, ATPases, PI-kinases, DNA methylases, metabolic enzymes, or protein glycosylases.

#### 25      5.1.2.      Recognition Units

By the phrase "recognition unit," is meant any molecule having a selective affinity for the functional domain of the target molecule and, preferably, having a molecular weight of up to about 20,000 daltons. In a particular embodiment of the invention, the recognition unit has a molecular weight that ranges from about 100 to about 10,000 daltons.

Accordingly, preferred recognition units of the present invention possess a molecular weight of about 100 to about 5,000 daltons, preferably from about 100 to about 2,000 daltons, and most preferably from about 500 to about 1,500 daltons. As described further below, the recognition unit of

the present invention can be a peptide, a carbohydrate, a nucleoside, an oligonucleotide, any small synthetic molecule, or a natural product. When the recognition unit is a peptide, the peptide preferably contains about 6 to about 60 amino acid residues.

When the recognition unit is a peptide, the peptide can have less than about 140 amino acid residues; preferably, the peptide has less than about 100 amino acid residues; preferably, the peptide has less than about 70 amino acid residues; preferably, the peptide has 20 to 50 amino acid residues; most preferably, the peptide has about 6 to 60 amino acid residues.

The peptide recognition units are preferably in the form of a multivalent peptide complex comprising avidin or streptavidin (optionally conjugated to a label such as alkaline phosphatase or horseradish peroxidase) and biotinylated peptides.

According to the present invention, a recognition unit (preferably in the form of a multivalent recognition unit complex) is used to screen a plurality of expression products of gene sequences containing nucleic acid sequences that are present in native RNA or DNA (e.g., cDNA library, genomic library).

The step of choosing a recognition unit can be accomplished in a number of ways that are known to those of ordinary skill, including but not limited to screening cDNA libraries or random peptide libraries for a peptide that binds to the functional domain of interest. See, e.g., Yu et al., 1994, Cell 76, 933-945; Sparks et al., 1994, J. Biol. Chem. 269, 23853-23856. Alternatively, a peptide or other small molecule or drug may be known to those of ordinary skill to bind to a certain target molecule and can be used. The recognition unit can even be synthesized from a lead compound, which again may be a peptide, carbohydrate, oligonucleotide, small drug molecule, or the like. The recognition unit can also be identified for use by doing searches (preferably via database) for molecules having homology for other, known

recognition unit(s) having the ability to selectively bind to the functional domain of interest.

In a specific embodiment, the step of selecting a recognition unit for use can be effected by, e.g., the use of  
5 diversity libraries, such as random or combinatorial peptide or nonpeptide libraries, which can be screened for molecules that specifically bind to the functional domain of interest, e.g., an SH3 domain. Many libraries are known in the art that can be used, e.g., chemically synthesized libraries,  
10 recombinant (e.g., phage display libraries), and *in vitro* translation-based libraries.

Examples of chemically synthesized libraries are described in Fodor et al., 1991, *Science* 251:767-773; Houghten et al., 1991, *Nature* 354:84-86; Lam et al., 1991,  
15 *Nature* 354:82-84; Medynski, 1994, *Bio/Technology* 12:709-710; Gallop et al., 1994, *J. Medicinal Chemistry* 37(9):1233-1251; Ohlmeyer et al., 1993, *Proc. Natl. Acad. Sci. USA* 90:10922-10926; Erb et al., 1994, *Proc. Natl. Acad. Sci. USA* 91:11422-11426; Houghten et al., 1992, *Biotechniques* 13:412;  
20 Jayawickreme et al., 1994, *Proc. Natl. Acad. Sci. USA* 91:1614-1618; Salmon et al., 1993, *Proc. Natl. Acad. Sci. USA* 90:11708-11712; PCT Publication No. WO 93/20242; and Brenner and Lerner, 1992, *Proc. Natl. Acad. Sci. USA* 89:5381-5383.

Examples of phage display libraries are described in  
25 Scott and Smith, 1990, *Science* 249:386-390; Devlin et al., 1990, *Science*, 249:404-406; Christian, R.B., et al., 1992, *J. Mol. Biol.* 227:711-718); Lenstra, 1992, *J. Immunol. Meth.* 152:149-157; Kay et al., 1993, *Gene* 128:59-65; and PCT Publication No. WO 94/18318 dated August 18, 1994.

30 *In vitro* translation-based libraries include but are not limited to those described in PCT Publication No. WO 91/05058 dated April 18, 1991; and Mattheakis et al., 1994, *Proc. Natl. Acad. Sci. USA* 91:9022-9026.

By way of examples of nonpeptide libraries, a  
35 benzodiazepine library (see e.g., Bunin et al., 1994, *Proc. Natl. Acad. Sci. USA* 91:4708-4712) can be adapted for use. Peptoid libraries (Simon et al., 1992, *Proc. Natl. Acad. Sci.*

USA 89:9367-9371) can also be used. Another example of a library that can be used, in which the amide functionalities in peptides have been permethylated to generate a chemically transformed combinatorial library, is described by Ostresh et al. (1994, Proc. Natl. Acad. Sci. USA 91:11138-11142).

The variety of non-peptide libraries that are useful in the present invention is great. For example, Ecker and Crooke, 1995, Bio/Technology 13:351-360 list benzodiazapines, hydantoins, piperazinediones, biphenyls, sugar analogs,  $\beta$ -mercaptoketones, arylacetic acids, acylpiperidines, benzopyrans, cubanes, xanthines, aminimides, and oxazolones as among the chemical species that form the basis of various libraries.

Non-peptide libraries can be classified broadly into two types: decorated monomers and oligomers. Decorated monomer libraries employ a relatively simple scaffold structure upon which a variety of functional groups is added. Often the scaffold will be a molecule with a known useful pharmacological activity. For example, the scaffold might be the benzodiazapine structure.

Non-peptide oligomer libraries utilize a large number of monomers that are assembled together in a ways that create new shapes that depend on the order of the monomers. Among the monomer units that have been used are carbamates, pyrrolinones, and morpholinos. Peptoids, peptide-like oligomers in which the side chain is attached to the  $\alpha$  amino group rather than the  $\alpha$  carbon, form the basis of another version of non-peptide oligomer libraries. The first non-peptide oligomer libraries utilized a single type of monomer and thus contained a repeating backbone. Recent libraries have utilized more than one monomer, giving the libraries added flexibility.

Screening the libraries can be accomplished by any of a variety of commonly known methods. See, e.g., the following references, which disclose screening of peptide libraries: Parmley and Smith, 1989, Adv. Exp. Med. Biol. 251:215-218; Scott and Smith, 1990, Science 249:386-390;

Fowlkes et al., 1992; BioTechniques 13:422-427; Oldenburg et al., 1992, Proc. Natl. Acad. Sci. USA 89:5393-5397; Yu et al., 1994, Cell 76:933-945; Staudt et al., 1988, Science 241:577-580; Bock et al., 1992, Nature 355:564-566; Tuerk et al.,  
5 1992, Proc. Natl. Acad. Sci. USA 89:6988-6992; Ellington et al., 1992, Nature 355:850-852; U.S. Patent No. 5,096,815, U.S. Patent No. 5,223,409, and U.S. Patent No. 5,198,346, all to Ladner et al.; Rebar and Pabo, 1993, Science 263:671-673; and PCT Publication No. WO 94/18318.

10 In a specific embodiment, screening to identify a recognition unit can be carried out by contacting the library members with an SH3 domain immobilized on a solid phase and harvesting those library members that bind to the SH3 domain. Examples of such screening methods, termed "panning"  
15 techniques are described by way of example in Parmley and Smith, 1988, Gene 73:305-318; Fowlkes et al., 1992, BioTechniques 13:422-427; PCT Publication No. WO 94/18318; and in references cited hereinabove.

In another embodiment, the two-hybrid system for  
20 selecting interacting proteins in yeast (Fields and Song, 1989, Nature 340:245-246; Chien et al., 1991, Proc. Natl. Acad. Sci. USA 88:9578-9582) can be used to identify recognition units that specifically bind to SH3 domains.

Where the recognition unit is a peptide, the peptide  
25 can be conveniently selected from any peptide library, including random peptide libraries, combinatorial peptide libraries, or biased peptide libraries. The term "biased" is used herein to mean that the method of generating the library is manipulated so as to restrict one or more parameters that  
30 govern the diversity of the resulting collection of molecules, in this case peptides.

Thus, a truly random peptide library would generate a collection of peptides in which the probability of finding a particular amino acid at a given position of the peptide is  
35 the same for all 20 amino acids. A bias can be introduced into the library, however, by specifying, for example, that a lysine occur every fifth amino acid or that positions 4, 8,

and 9 of a decapeptide library be fixed to include only arginine. Clearly, many types of biases can be contemplated, and the present invention is not restricted to any particular bias. Furthermore, the present invention contemplates  
5 specific types of peptide libraries, such as phage-displayed peptide libraries and those that utilize a DNA construct comprising a lambda phage vector with a DNA insert.

As mentioned above, in the case of a recognition unit that is a peptide, the peptide may have about 6 to less  
10 than about 60 amino acid residues, preferably about 6 to about 25 amino acid residues, and most preferably, about 6 to about 15 amino acids. In another embodiment, a peptide recognition unit has in the range of 20-100 amino acids, or 20-50 amino acids. In the case of a bile acid receptor, for example, the  
15 recognition unit may be a bile acid, such as cholic acid or cholesterol, and may have a molecular weight of about 300 to about 600. If the functional domain relates to transcriptional control, the recognition unit may be a portion of a transcriptional factor, which may bind to a region of a  
20 gene of interest or to an RNA polymerase. The recognition unit may even be a nucleoside analog, such as cordycepin or the triphosphate thereof, capable of inhibiting RNA biosynthesis. The recognition unit may also be the carbohydrate portion of a glycoprotein, which may have a  
25 selective affinity for the asialoglycoprotein receptor, or the repeating glucan unit that exhibits a selective affinity for a cellulose binding domain or the active site of heparinase.

The selected recognition unit can be obtained by chemical synthesis or recombinant expression. It is  
30 preferably purified prior to use in screening a plurality of gene sequences.

#### 5.1.3. Screening a Source of Polypeptides

After the recognition unit is chosen for use, the  
35 recognition unit is then contacted with a plurality of polypeptides, preferably containing a functional domain. In a particular embodiment of the invention, the plurality of

polypeptides is obtained from a polypeptide expression library. The polypeptide expression library may be obtained, in turn, from cDNA, fragmented genomic DNA, and the like. In a specific embodiment, the library that is screened is a cDNA library of total poly A+ RNA of an organism, in general, or of a particular cell or tissue type or developmental stage or disease condition or stage. The expression library may utilize a number of expression vehicles known to those of ordinary skill, including but not limited to, recombinant bacteriophage, lambda phage, M13, a recombinant plasmid or cosmid, and the like.

The plurality of polypeptides or the DNA sequences encoding same may be obtained from a variety of natural or unnatural sources, such as a procaryotic or a eucaryotic cell, either a wild type, recombinant, or mutant. In particular, the plurality of polypeptides may be endogenous to microorganisms, such as bacteria, yeast, or fungi, to a virus, to an animal (including mammals, invertebrates, reptiles, birds, and insects) or to a plant cell.

In addition, the plurality of polypeptides may be obtained from more specific sources, such as the surface coat of a virion particle, a particular cell lysate, a tissue extract, or they may be restricted to those polypeptides that are expressed on the surface of a cell membrane.

Moreover, the plurality of polypeptides may be obtained from a biological fluid, particularly from humans, including but not limited to blood, plasma, serum, urine, feces, mucus, semen, vaginal fluid, amniotic fluid, or cerebrospinal fluid. The plurality of polypeptides may even be obtained from a fermentation broth or a conditioned medium, including all the polypeptide products secreted or produced by the cells previously in the broth or medium.

The step of contacting the recognition unit with the plurality of polypeptides may be effected in a number of ways. For example, one may contemplate immobilizing the recognition unit on a solid support and bringing a solution of the plurality of polypeptides in contact with the immobilized



recognition unit. Such a procedure would be akin to an affinity chromatographic process, with the affinity matrix being comprised of the immobilized recognition unit. The polypeptides having a selective affinity for the recognition unit can then be purified by affinity selection. The nature of the solid support, process for attachment of the recognition unit to the solid support, solvent, and conditions of the affinity isolation or selection procedure would depend on the type of recognition unit in use but would be largely conventional and well known to those of ordinary skill in the art. Moreover, the valency of the recognition unit in the recognition unit complex used to screen the polypeptides is believed to affect the specificity of the screening step, and thus the valency can be chosen as appropriate in view of the desired specificity (see Sections 5.2 and 5.2.1).

Alternatively, one may also separate the plurality of polypeptides into substantially separate fractions comprising individual polypeptides. For instance, one can separate the plurality of polypeptides by gel electrophoresis, column chromatography, or like method known to those of ordinary skill for the separation of polypeptides. The individual polypeptides can also be produced by a transformed host cell in such a way as to be expressed on or about its outer surface. Individual isolates can then be "probed" by the recognition unit, optionally in the presence of an inducer should one be required for expression, to determine if any selective affinity interaction takes place between the recognition unit and the individual clone. Prior to contacting the recognition unit with each fraction comprising individual polypeptides, the polypeptides can optionally first be transferred to a solid support for additional convenience. Such a solid support may simply be a piece of filter membrane, such as one made of nitrocellulose or nylon.

In this manner, positive clones can be identified from a collection of transformed host cells of an expression library, which harbor a DNA construct encoding a polypeptide having a selective affinity for the recognition unit. The

polypeptide produced by the positive clone includes the functional domain of interest or a functional equivalent thereof. Furthermore, the amino acid sequence of the polypeptide having a selective affinity for the recognition unit can be determined directly by conventional means of amino acid sequencing, or the coding sequence of the DNA encoding the polypeptide can frequently be determined more conveniently by use of standard DNA sequencing methods. The primary sequence can then be deduced from the corresponding DNA sequence.

If the amino acid sequence is to be determined from the polypeptide itself, one may use microsequencing techniques. The sequencing technique may include mass spectroscopy.

In certain situations, it may be desirable to wash away any unbound recognition unit from a mixture of the recognition unit and the plurality of polypeptides prior to attempting to determine or to detect the presence of a selective affinity interaction (i.e., the presence of a recognition unit that remains bound after the washing step). Such a wash step may be particularly desirable when the plurality of polypeptides is bound to a solid support.

As can be anticipated, the degree of selective affinities observed varies widely, generally falling in the range of about 1 nM to about 1 mM. In preferred embodiments of the present invention, the selective affinity is on the order of about 10 nM to about 100  $\mu$ M, more preferably on the order of about 100 nM to about 10  $\mu$ M, and most preferably on the order of about 100 nM to about 1  $\mu$ M.

## 5.2. Specificity of Recognition Units

A particular recognition unit may have fairly generic selectivity for a several members (e.g., three or four or more) of a "panel" of polypeptides having the domain of interest (or different versions of the domain of interest or functional equivalents of the domain of interest) or a fairly specific selectivity for only one or two, or possibly three,

of the polypeptides among a "panel" of same. Furthermore, multiple recognition units, each exhibiting a range of selectivities among a "panel" of polypeptides can be used to identify an increasingly comprehensive set of additional  
5 polypeptides that include the functional domain of interest.

Hence, in a population of related polypeptides, the functional domains of interest of each member may be schematically represented by a circle. See, by way of example, Figure 7A. The circle of one polypeptide may overlap  
10 with that of another polypeptide. Such overlaps may be few or numerous for each polypeptide. A particular recognition unit, A, may recognize or interact with a portion of the circle of a given polypeptide which does not overlap with any other circle. Such a recognition unit would be fairly specific to  
15 that polypeptide. On the other hand, a second recognition unit, B, may recognize a region of overlap between two or more polypeptides. Such a recognition unit would consequently be less specific than the recognition unit A and may be characterized as having a more generic specificity depending  
20 on the number of polypeptides that it recognizes or interacts with.

It should also be apparent to those of ordinary skill that any number of B-type recognition units ( $B_1$ ,  $B_2$ ,  $B_3$ , etc.) can be present, each recognizing different "panels" of  
25 polypeptides. Hence, the use of multiple recognition units provides an increasingly more exhaustive population of polypeptides, each of which exhibits a variation or evolution in the functional domain of interest present in the initial target molecule. It should also be apparent to one that the  
30 present method can be applied in an iterative fashion, such that the identification of a particular polypeptide can lead to the choice of another recognition unit. See, e.g., Figure 7B. Use of this new recognition unit will lead, in turn, to the identification of other polypeptides that contain  
35 functional domains of interest that enhance the phenotypic and/or genotypic diversity of the population of "related" polypeptides.

Hence, with a given recognition unit, one may observe interaction with only one or two different polypeptides. With other recognition units, one may find three, four, or more selective interactions. In the situation  
5 in which only a single interaction is observed, it is likely, though not mandatory, that the selective affinity interaction is between the recognition unit and a replica of the initial target molecule (or a molecule very similar structurally and "functionally" to the initial target molecule).

10

5.2.1. Effect of the Presentation of the Recognition Unit Complex on the Specificity of the  
Recognition Unit-Functional Domain Interaction

The present inventors have found, unexpectedly, that the valency (i.e., whether it is a monomer, dimer, tetramer,  
15 etc.) of the recognition unit that is used to screen an expression library or other source of polypeptides apparently has a marked effect upon which genes or polypeptides are identified from the expression library or source of polypeptides. In particular, the specificity of the  
20 recognition unit-functional domain interaction appears to be affected by the valency of the recognition unit in the screening process. By this specificity is meant the selectivity in the functional domains to which the recognition unit will bind in the screening step.

25

As discussed above, in one embodiment, recognition units are obtained by screening a source of recognition units, e.g., a phage display library, for recognition units that bind to a particular target functional domain. Alternatively, database searches for recognition units with sequence homology  
30 to known recognition units can be employed. Of course, if a recognition unit for a particular target functional domain is already known, there is no need to screen a library or other source of recognition units; one can merely synthesize that particular recognition unit. The recognition unit, however  
35 obtained, is then used to screen an expression library or other source of polypeptides, to identify polypeptides that

the recognition unit binds to. A recognition unit that identifies only its target functional domain is a recognition unit that is completely specific. A recognition unit that identifies one or two other polypeptides that do not contain 5 identically the target functional domain, from among a plurality of polypeptides (e.g., of greater than  $10^4$ ,  $10^6$ , or  $10^8$  complexity), in addition to identifying a molecule comprising its target functional domain, is very or highly specific. A recognition unit that identifies most other 10 polypeptides present that do not contain its target functional domain, in addition to identifying its target functional domain, is a non-specific recognition unit. In between very specific recognition units and non-specific recognition units, the present inventors have discovered that there are 15 recognition units that recognize a small number of molecules having functional domains other than their target functional domains. These recognition units are said to have generic specificity.

Thus, there is a "specificity continuum", from 20 completely and very specific through generic to non-specific, that a recognition unit may evince. See Figure 11 for a depiction of this specificity continuum. The Applicants have discovered that a major factor influencing the specificity exhibited by a recognition unit appears to be the valency of 25 the recognition unit in the complex used to screen the expression library.

Usually, high specificity is considered to be desirable when screening a library. High specificity is exhibited, e.g., by affinity purified polyclonal antisera 30 which, in general, are very specific. Monoclonal antibodies are also very specific. Small peptides in monovalent form, on the other hand, generally give very weak, non-specific signals when used to screen a library; thus, they are considered to be non-specific.

35 The present inventors have discovered that recognition units in the form of small peptides, in multivalent form, have a specificity midway between the high

specificity of antibodies and the low/non-specificity of monovalent peptides. Multivalency of the recognition unit of at least two, in a recognition unit complex used to screen the gene library, is preferred, with a multivalency of at least 5 four more preferred, to obtain a screening wherein specificity is eased but not forfeited. In particular, a multivalent (believed to be tetravalent) recognition unit complex comprising streptavidin or avidin (preferably conjugated to a label, e.g., an enzyme such as alkaline phosphatase or 10 horseradish peroxidase, or a fluorogen, e.g. green fluorescent protein) and biotinylated peptide recognition units have an unexpected generic specificity. This allows such peptides to be used to screen libraries to identify classes of polypeptides containing functional domains that are similar 15 but not identical to the peptides' target functional domains. These classes of polypeptides are identified despite the low level of homology at the amino acid level of the functional domains of the members of the classes.

In another specific embodiment, multivalent peptide 20 recognition units may be in the form of multiple antigen peptides (MAP) (Tam, 1989, J. Imm. Meth. 124:53-61; Tam, 1988, Proc. Natl. Acad. Sci. USA 85:5409-5413). In this form, the peptide recognition unit is synthesized on a branching lysyl matrix using solid-phase peptide synthesis methods. 25 Recognition units in the form of MAP may be prepared by methods known in the art (Tam, 1989, J. Imm. Meth. 124:53-61; Tam, 1988, Proc. Natl. Acad. Sci. USA 85:5409-5413), or, for example, by a stepwise solid-phase procedure on MAP resins (Applied Biosystems), utilizing methodology established by the 30 manufacturer. MAP peptides may be synthesized comprising (recognition unit peptide)<sub>2</sub>Lys<sub>1</sub>, (recognition unit peptide)<sub>4</sub>Lys<sub>3</sub>, (recognition unit peptide)<sub>8</sub>Lys<sub>6</sub> or more levels of branching.

The multivalent peptide recognition unit complexes 35 may also be prepared by cross-linking the peptide to a carrier protein, e.g., bovine serum albumin (BSA), keyhole limpet hemocyanin (KLH), or an enzyme, by use of known cross-linking

reagents. Such cross-linked peptide recognition units may be detected by, e.g., an antibody to the carrier protein or detection of the enzymatic activity of the carrier protein.

Furthermore, the present inventors have discovered  
5 what specificity is exhibited by various types of recognition units and their complexes, i.e., where these recognition units and their complexes fall in the specificity continuum. The present inventors have discovered a range of formats for presenting recognition units used to screen libraries. For  
10 example, the present inventors have determined that a peptide in the form of a bivalent fusion protein with alkaline phosphatase is very specific. The same peptide in the form of a fusion protein with the pIII protein of an M13 derived bacteriophage, expressed on the phage surface, has somewhat  
15 less, though still high, specificity. That same peptide when biotinylated in the form of a tetravalent streptavidin-alkaline phosphatase complex has generic specificity. Use of such a generically specific peptide permits the identification of a wide range of proteins from expression libraries or other  
20 sources of polypeptides, each protein containing an example of a particular functional domain.

Accordingly, the present invention provides a method of modulating the specificity of a peptide such that the peptide can be used as a recognition unit to screen a  
25 plurality of polypeptides, thus identifying polypeptides that have a functional domain. In a specific embodiment, specificity is generic so as to provide for the identification of polypeptides having a functional domain that varies in sequence from that of the target functional domain known to  
30 bind the recognition unit under conditions of high specificity. In a particular embodiment, the method comprises forming a tetravalent complex of the biotinylated peptide and streptavidin-alkaline phosphatase prior to use for screening an expression library.

35

### 5.3. Kits

The present invention is also directed to an assay kit which can be useful in the screening of drug candidates. In a particular embodiment of the present invention, an assay  
5 kit is contemplated which comprises in one or more containers (a) a polypeptide containing a functional domain of interest; and (b) a recognition unit having a selective affinity for the polypeptide. The kit optionally further comprises a detection means for determining the presence of a polypeptide-  
10 recognition unit interaction or the absence thereof.

In a specific embodiment, either the polypeptide containing the functional domain or the recognition unit is labeled. A wide range of labels can be used to advantage in the present invention, including but not limited to  
15 conjugating the recognition unit to biotin by conventional means. Alternatively, the label may comprise a fluorogen, an enzyme, an epitope, a chromogen, or a radionuclide. Preferably, the biotin is conjugated by covalent attachment to either the polypeptide or the recognition unit. The  
20 polypeptide or, preferably, the recognition unit is immobilized on a solid support. The detection means employed to detect the label will depend on the nature of the label and can be any known in the art, e.g., film to detect a radionuclide; an enzyme substrate that gives rise to a  
25 detectable signal to detect the presence of an enzyme; antibody to detect the presence of an epitope, etc.

A further embodiment of the assay kit of the present invention includes the use of a plurality of polypeptides, each polypeptide containing a functional domain of interest.  
30 The assay kit further comprises at least one recognition unit having a selective affinity for each of the plurality of polypeptides and a detection means for determining the presence of a polypeptide-recognition unit interaction or the absence thereof.

35 A kit is provided that comprises, in one or more containers, a first molecule comprising an SH3 domain and a second molecule that binds to the SH3 domain, i.e., a



recognition unit, where the SH3 domain is a novel SH3 domain identified by the methods of the present invention.

In a specific embodiment, the present invention provides an assay kit comprising in one or more containers:

- 5 (a) a purified polypeptide containing a functional domain of interest, in which the functional domain of is a domain selected from the group consisting of an SH1, SH2, SH3, PH, PTB, LIM, armadillo, Notch/ankyrin repeat, zinc finger, leucine zipper, and helix-turn-helix; and
- 10 (b) a purified recognition unit having a selective binding affinity for said functional domain in said polypeptide.

In the above assay kit, the polypeptide may comprise an amino acid sequence selected from the group consisting of  
15 SEQ ID NOS: 8, 10, 12, 18, 20, 22, 24, 30, 32, 38, 40, 190, 192, 194, 196, 198, 200, 221, 113-115, 118-121, 125-128, 133-139, 204-218, and 219.

In the above assay kit, the polypeptide may comprise an amino acid sequence selected from the group consisting of  
20 SEQ ID NOS: 6, 14, 16, 26, 28, 34, 36, 112, 116, 117, 122-124, 129-132, and 140.

In other embodiments of the above-described assay kit, the recognition unit may be a peptide. The recognition unit may be labeled with e.g., an enzyme, an epitope, a  
25 chromogen, or biotin.

In another specific embodiment, the present invention provides an assay kit comprising in containers:

- (a) a plurality of purified polypeptides, each polypeptide in a separate container and each polypeptide  
30 containing a functional domain of interest in which the functional domain of interest is a domain selected from the group consisting of an SH1, SH2, SH3, PH, PTB, LIM, armadillo, Notch/ankyrin repeat, zinc fingers, leucine zippers, and helix-turn-helix; and
- 35 (b) at least one recognition unit having a selective binding affinity for said functional domain in each of said plurality of polypeptides.

The present invention also provides an assay kit comprising in one or more containers:

(a) a plurality of purified polypeptides, each polypeptide in a separate container and each polypeptide  
5 containing an SH3 domain; and

(b) at least one peptide having a selective affinity for the SH3 domain in each of said plurality of polypeptides.

The present invention also provides a kit comprising  
10 a plurality of purified polypeptides comprising a functional domain of interest, each polypeptide in a separate container, and each polypeptide having a functional domain of a different sequence but capable of displaying the same binding specificity.

15 In the above-described kits, the polypeptides may have an amino acid sequence selected from the group consisting of: SEQ ID NOS:8, 10, 12, 18, 20, 22, 24, 30, 32, 38, 40, 190, 192, 194, 196, 198, 200, 221.

In the above-described kits, the functional domain  
20 may be an SH3 domain.

The molecular components of the kits are preferably purified.

The kits of the present invention may be used in the methods for identifying new drug candidates and determining  
25 the specificities thereof that are described in Section 5.4.

#### 5.4. Assays for the Identification of Potential Drug Candidates and Determining the Specificity Thereof

The present invention also provides methods for  
30 identifying potential drug candidates (and lead compounds) and determining the specificities thereof. For example, knowing that a polypeptide with a functional domain of interest and a recognition unit, e.g., a binding peptide, exhibit a selective affinity for each other, one may attempt to identify a drug  
35 that can exert an effect on the polypeptide-recognition unit interaction, e.g., either as an agonist or as an antagonist (inhibitor) of the interaction. With this assay, one can

screen a collection of candidate "drugs" for the one exhibiting the most desired characteristic, e.g., the most efficacious in disrupting the interaction or in competing with the recognition unit for binding to the polypeptide.

5 Alternatively, one may utilize the different selectivities that a particular recognition unit may exhibit for different polypeptides bearing the same, similar, or functionally equivalent functional domains. Thus, one may tailor the screen to identify drug candidates that exhibit  
10 more selective activities directed to specific polypeptide-recognition unit interactions, among the "panel" of possibilities. Thus, for example, a drug candidate may be screened to identify the presence or absence of an effect on particular binding interactions, potentially leading to  
15 undesirable side effects.

Indeed, an intriguing application of the present invention is described as follows. A known antiviral agent, FIAU (a halogenated nucleoside analog), is effective at given dosages against the virus that causes hepatitis B. This  
20 compound is suspected of causing toxic side effects, however, which give rise to liver failure in certain patients to whom the drug is administered. According to the present invention, an assay is provided which can be used to develop a new generation of FIAU-derived drug that maintains its  
25 effectiveness against viral replication while reducing liver toxicity. Such an assay is provided by choosing FIAU as a recognition unit having a selective affinity for a polypeptide present in the hepatitis B virus or a cell infected with the virus. This polypeptide or family of polypeptides having the  
30 functional domain of interest is obtained by allowing the chosen recognition unit, FIAU, to come into contact with an expression library comprised of the hepatitis B virus genome and/or a cDNA expression library of infected cells, according to the methods of the present invention.

35 Likewise, the chosen recognition unit is allowed to come into contact with a plurality of polypeptides obtained from a sample of a human liver extract or of noninfected

hepatocytes. In this manner, a "panel" of polypeptides each of which exhibits a selective affinity for the chosen recognition unit is identified. As described above, this panel is used to determine the activities of drug (FIAU) homologs, analogs, or derivatives in terms of, say, selective inhibition of viral polypeptide-FIAU interaction versus liver polypeptide-FIAU interaction. Hence, those drug homologs, analogs, or derivatives that maintain a selective affinity for the viral polypeptide (or infected cell polypeptide) while failing to interact with or having a minimal binding affinity for liver polypeptides (and, hence, have reduced toxicity in the liver due to elimination of undesirable molecular interactions) can be identified and selected. Additional iterations of this process can be performed if so desired.

Therefore, the present invention contemplates an assay for screening a drug candidate comprising: (a) allowing at least one polypeptide comprising a functional domain of interest to come into contact with at least one recognition unit having a selective affinity for the polypeptide in the presence of an amount of a drug candidate, such that the polypeptide and the recognition unit are capable of interacting when brought into contact with one another in the absence of said drug candidate, and in which the functional domain of interest is a domain selected from the group consisting of an SH1, SH2, SH3, PH, PTB, LIM, armadillo, Notch/ankyrin repeat, zinc finger, leucine zipper, and helix-turn-helix; and (b) determining the effect, if any, of the presence of the amount of the drug candidate on the interaction of the polypeptide with the recognition unit.

In one embodiment, the effect of the drug candidate upon multiple, different interacting polypeptide-recognition unit pairs is determined in which at least some of said polypeptides have a functional domain that differs in sequence but is capable of displaying the same binding specificity as the functional domain in another of said polypeptides.

In another embodiment, at least one of said at least one polypeptide or recognition unit contains a consensus

functional domain and consensus recognition unit, respectively.

In another embodiment, the drug candidate is an inhibitor of the polypeptide-recognition unit interaction that  
5 is identified by detecting a decrease in the binding of polypeptide to recognition unit in the presence of such inhibitor.

In another embodiment, said polypeptide is a polypeptide containing an SH3 domain produced by a method  
10 comprising:

- (i) screening a peptide library with an SH3 domain to obtain one or more peptides that bind the SH3 domain;
- (ii) using one of the peptides from step (i) to screen a source of polypeptides to identify one or more  
15 polypeptides containing an SH3 domain;
- (iii) determining the amino acid sequence of the polypeptides identified in step (ii); and
- (iv) producing the one or more novel polypeptides containing an SH3 domain.

20 In another embodiment, said polypeptide is a polypeptide containing an SH3 domain produced by a method comprising:

- (i) screening a peptide library with an SH3 domain to obtain a plurality of peptides that bind the SH3 domain;
- 25 (ii) determining a consensus sequence for the peptides obtained in step (i);
- (iii) producing a peptide comprising the consensus sequence;
- (iv) using the peptide comprising the consensus  
30 sequence to screen a source of polypeptides to identify one or more polypeptides containing an SH3 domain;
- (v) determining the amino acid sequence of the polypeptides identified in step (iv); and
- (vi) producing the one or more polypeptides  
35 containing an SH3 domain.

In a preferred embodiment, the effect of the drug candidate upon multiple, different interacting polypeptide-

recognition unit pairs is determined in which preferably at least some (e.g., at least 2, 3, 4, 5, 7, or 10) of said polypeptides have functional domains that vary in sequence yet are capable of displaying the same binding specificity, i.e., binding to the same recognition unit. In another specific embodiment, at least one of said polypeptides and/or recognition units contain a consensus functional domain and recognition unit, respectively (and thus are not known to be naturally expressed proteins). In one embodiment, the polypeptide is a novel polypeptide identified by the methods of the present invention. In a specific embodiment, an inhibitor of the polypeptide-recognition unit interaction is identified by detecting a decrease in the binding of polypeptide to recognition unit in the presence of such inhibitor.

A common problem in the development of new drugs is that of identifying a single, or a small number, of compounds that possess a desirable characteristic from among a background of a large number of compounds that lack that desired characteristic. This problem arises both in the testing of compounds that are natural products from plant, animal, or microbial sources and in the testing of man-made compounds. Typically, hundreds, or even thousands, of compounds are randomly screened by the use of *in vitro* assays such as those that monitor the compound's effect on some enzymatic activity, its ability to bind to a reference substance such as a receptor or other protein, or its ability to disrupt the binding between a receptor and its ligand.

The compounds which pass this original screening test are known as "lead" compounds. These lead compounds are then put through further testing, including, eventually, *in vivo* testing in animals and humans, from which the promise shown by the lead compounds in the original *in vitro* tests is either confirmed or refuted. See Remington's Pharmaceutical Sciences, 1990, A.R. Gennaro, ed., Chapter 8, pages 60-62, Mack Publishing Co., Easton, PA; Ecker and Crooke, 1995, *Bio/Technology* 13:351-360.

There is a continual need for new compounds to be tested in the *in vitro* assays that make up the first testing step described above. There is also a continual need for new assays by which the pharmacological activities of these compounds may be tested. It is an object of the present invention to provide such new assays to determine whether a candidate compound is capable of affecting the binding between a polypeptide containing a functional domain and a recognition unit that binds to that functional domain. In particular, it is an object of the present invention to provide polypeptides, particularly novel ones, containing functional domains and their corresponding recognition units for use in the above-described assays. The use of these polypeptides greatly expands the number of assays that may be used to screen potential drug candidates for useful pharmacological activities (as well as to identify potential drug candidates that display adverse or undesirable pharmacological activities). In one particular embodiment of the present invention, the polypeptides contain an SH3 domain.

In one embodiment of the present invention, such polypeptides are identified by a method comprising: using a recognition unit that is capable of binding to a predetermined functional domain to screen a source of polypeptides, thus identifying novel polypeptides containing the functional domain or a similar functional domain.

In a particular embodiment of the above-described method, the novel polypeptide comprises an SH3 domain and is obtained by:

- (i) screening a peptide library with the SH3 domain to obtain one or more peptides that bind the SH3 domain;
- (ii) using one of the peptides from step (i), preferably in the form of a multivalent complex, to screen a source of polypeptides to identify one or more novel polypeptides containing SH3 domains;
- (iii) determining the amino acid sequence of the polypeptides identified in step (ii); and

(iv) producing the one or more novel polypeptides containing SH3 domains.

In another embodiment of the above-described method, the novel polypeptide containing an SH3 domain is obtained by:

- 5 (i) screening a peptide library with the SH3 domain to obtain peptides that bind the SH3 domain;
- (ii) determining a consensus sequence for the peptides obtained in step (i);
- (iii) producing a peptide comprising the consensus  
10 sequence;
- (iv) using the peptide comprising the consensus sequence to screen a source of polypeptides to identify one or more novel polypeptides containing SH3 domains;
- (v) determining the amino acid sequence of the novel  
15 polypeptides identified in step (iv); and
- (vi) producing the one or more novel polypeptides containing SH3 domains.

One of ordinary skill in the art will recognize that it will not always be necessary to utilize the entire novel  
20 polypeptide containing the SH3 domain in the assays described herein. Often, a portion of the polypeptide that contains the SH3 domain will be sufficient, e.g., a glutathione S-transferase (GST)-SH3 domain fusion protein. See Figure 10A and 10B for a depiction of the portions of the exemplary novel  
25 polypeptides that contain SH3 domains.

A typical assay of the present invention consists of at least the following components: (1) a molecule (e.g., protein or polypeptide) comprising a functional domain; (2) a recognition unit that selectively binds to the functional  
30 domain; (3) a candidate compound, suspected of having the capacity to affect the binding between the protein containing the functional domain and the recognition unit. The assay components may further comprise (4) a means of detecting the binding of the protein comprising the functional domain and  
35 the recognition unit. Such means can be e.g., a detectable label affixed to the protein comprising the functional domain, the recognition unit, or the candidate compound.



In a specific embodiment, the protein comprising the functional domain is a novel protein discovered by the methods of the present invention.

In another specific embodiment, the invention provides a method of identifying a compound that affects the binding of a molecule comprising a functional domain and a recognition unit that selectively binds to the functional domain comprising:

(a) contacting the molecule comprising the functional domain and the recognition unit under conditions conducive to binding in the presence of a candidate compound and measuring the amount of binding between the molecule and the recognition unit;

(b) comparing the amount of binding in step (a) with the amount of binding known or determined to occur between the molecule and the recognition unit in the absence of the candidate compound, where a difference in the amount of binding between step (a) and the amount of binding known or determined to occur between the molecule and the recognition unit in the absence of the candidate compound indicates that the candidate compound is a compound that affects the binding of the molecule comprising a functional domain and the recognition unit. In a specific embodiment, the molecule comprising the functional domain is a novel protein discovered by the methods of the present invention. In another specific embodiment, the functional domain is an SH3 domain.

In one embodiment, the assay comprises allowing the polypeptide containing an SH3 domain to contact a recognition unit that selectively binds to the SH3 domain in the presence and in the absence of the candidate compound under conditions such that binding of the recognition unit to the protein containing an SH3 domain will occur unless that binding is disrupted or prevented by the candidate compound. By detecting the amount of binding of the recognition unit to the protein containing an SH3 domain in the presence of the candidate compound and comparing that amount of binding to the amount of binding of the recognition unit to the protein or

polypeptide containing an SH3 domain in the absence of the candidate compound, it is possible to determine whether the candidate compound affects the binding and thus is a useful lead compound for the modulation of the activity of proteins  
5 containing the SH3 domain. The effect of the candidate compound may be to either increase or decrease the binding.

One version of an assay suitable for use in the present invention comprises binding the protein containing an SH3 domain to a solid support such as the wells of a  
10 microtiter plate. The wells contain a suitable buffer and other substances to ensure that conditions in the wells permit the binding of the protein or polypeptide containing an SH3 domain to its recognition unit. The recognition unit and a candidate compound are then added to the wells. The  
15 recognition unit is preferably labeled, e.g., it might be biotinylated or labeled with a radioactive moiety, or it might be linked to an enzyme, e.g., alkaline phosphatase. After a suitable period of incubation, the wells are washed to remove any unbound recognition unit and compound. If the candidate  
20 compound does not interfere with the binding of the protein or polypeptide containing an SH3 domain to the labeled recognition unit, the labeled recognition unit will bind to the protein or polypeptide containing an SH3 domain in the well. This binding can then be detected. If the candidate  
25 compound interferes with the binding of the protein or polypeptide containing an SH3 domain and the labeled recognition unit, label will not be present in the wells, or will be present to a lesser degree than is the case when compared to control wells that contain the protein or  
30 polypeptide containing an SH3 domain and the labeled recognition unit but to which no candidate compound is added. Of course, it is possible that the presence of the candidate compound will increase the binding between the protein or polypeptide containing an SH3 domain and the labeled  
35 recognition unit. Alternatively, the recognition unit can be affixed to a solid substrate during the assay. Functional

domains other than SH3 domains and their corresponding recognition units can also be used.

In a specific embodiment of the above-described method, the protein or polypeptide containing an SH3 domain is a novel protein or polypeptide containing an SH3 domain that has been identified by the methods of the present invention.

#### 5.5. Use of Polypeptides Containing Functional Domains to Discover Polypeptides Involved in Pharmacological Activities

Using the methods of the present invention, it is possible to identify and isolate large numbers of polypeptides containing functional domains, e.g., SH3 domains. Using these polypeptides, one can construct a matrix relating the polypeptides to an array of candidate drug compounds. For example, Table 1 shows such a matrix.

TABLE 1

	A	B	C	D	E	F	G	H	I	J
1										
2		X		X				X		
3										
4										
5						X				
6										
7			X					X		
8										
9	X									
10										

In Table 1, the columns headed by letters at the top of the table represent different polypeptides containing SH3 domains (preferably novel polypeptides identified by the methods of the invention). The rows numbered along the left side of the table represent recognition units with various specificity to SH3 domains. For each candidate drug compound,

a table such as Table 1 is generated from the results of binding assays. An X placed at the intersection of a particular numbered row and lettered column represents a positive assay for binding, i.e., the candidate drug compound 5 affected the binding of the recognition unit of that particular row to the SH3 domain of that particular column.

Such data as that illustrated above is used to determine whether candidate drug compounds display or are at risk of displaying desirable or undesirable physiological or 10 pharmacological activities. For example, in Table 1, the drug compound inhibits the binding of recognition unit 2 to the SH3 domains of polypeptides B, D, and H; the compound inhibits the binding of recognition unit 5 to the SH3 domain of polypeptide F; the compound inhibits the binding of recognition unit 7 to 15 the SH3 domains of polypeptides C and H; and the compound inhibits the binding of recognition unit 9 to the SH3 domain of polypeptide A.

If interaction with polypeptide H leads to the desirable physiological or pharmacological activity, then this 20 drug candidate might be a good lead. However, interaction with polypeptides A, B, C, D, and F would need to be evaluated for potential side effects.

As the maps are generated and pharmacological effects observed, the maps will allow strategic assessment of 25 the specificity necessary to obtain the desired pharmacological effect. For example, if compounds 2 and 7 are able to affect some pharmacological activity, while compounds 5 and 9 do not affect that activity, then polypeptide H is likely to be involved in that pharmacological activity. For 30 example, if compounds 2 and 7 were both able to inhibit mast cell degranulation, while compounds 5 and 9 did not, it is likely that polypeptide H is involved in mast cell degranulation.

Accordingly, the present invention provides a method 35 of utilizing the polypeptides comprising functional domains of the present invention in an assay to determine the participation of those polypeptides in pharmacological

activities. In a particular embodiment, the polypeptides comprise SH3 domains.

In another embodiment, the method comprises:

(a) contacting a drug candidate with a molecule  
5 comprising a functional domain under conditions conducive to binding, and detecting or measuring any specific binding that occurs; and

(b) repeating step (a) with a plurality of different molecules, each comprising a different functional domain but  
10 capable of binding to a single predetermined recognition unit under appropriate conditions.

Preferably, at least one of said molecules is a novel polypeptide identified by the methods of the present invention. In a specific embodiment, the molecules comprise  
15 the SH3 domains of Src, Abl, Cortactin, Phospholipase C $\gamma$ , Nck, Crk, p53bp2, Amphiphysin, Grb2, RasGap, or Phosphatidylinositol 3' kinase.

The present invention also provides a method of determining the potential pharmacological activities of a  
20 molecule comprising:

(a) contacting the molecule with a compound comprising a functional domain under conditions conducive to binding;

(b) detecting or measuring any specific binding that  
25 occurs; and

(c) repeating steps (a) and (b) with a plurality of different compounds, each compound comprising a functional domain of different sequence but capable of displaying the same binding specificity.

30 In a specific embodiment the functional domain is an SH3 domain.

In another embodiment, the compounds comprise the SH3 domains of Src, Abl, Cortactin, Phospholipase C $\gamma$ , Nck, Crk, p53bp2, Amphiphysin, Grb2, RasGap, or Phosphatidylinositol 3' kinase.  
35

The present invention also provides a method of identifying a compound that affects the binding of a molecule

comprising a functional domain to a recognition unit that selectively binds to the functional domain comprising:

(a) contacting the molecule comprising the functional domain and the recognition unit under conditions conducive to binding in the presence of a candidate compound and measuring the amount of binding between the molecule and the recognition unit and in which the functional domain of interest is a domain selected from the group consisting of an SH1, SH2, SH3, PH, PTB, LIM, armadillo, Notch/ankyrin repeat, zinc finger, leucine zipper, and helix-turn-helix;

(b) comparing the amount of binding in step (a) with the amount of binding known or determined to occur between the molecule and the recognition unit in the absence of the candidate compound, where a difference in the amount of binding between step (a) and the amount of binding known or determined to occur between the molecule and the recognition unit in the absence of the candidate compound indicates that the candidate compound is a compound that affects the binding of the molecule comprising a functional domain and the recognition unit.

In a specific embodiment, the functional domain is an SH3 domain.

#### 5.6. Use of More Than One Recognition Unit Simultaneously

It has been found that when screening a source of polypeptides with a recognition unit, it is possible to use more than one recognition unit at the same time. In particular, it has been found that as many as five different recognition units may be used simultaneously to screen a source of polypeptides.

In particular, when the recognition units are biotinylated peptides and the source of polypeptides is a cDNA expression library, the steps of preconjugation of the biotinylated peptides to streptavidin-alkaline phosphatase as well as the steps involved in screening the cDNA expression library may be carried out in essentially the same manner as is done when a single biotinylated peptide is used as a

recognition unit. See Section 6.1 for details. The key difference when using more than one biotinylated peptide at a time is that the peptides are combined either before or at the step where they are placed in contact with the polypeptides from which selection occurs.

In an embodiment employing a bacteriophage expression library to express the polypeptides, when the positive clones are worked up to the level of isolated plaques, the clonal bacteriophage from the isolated plaques may be tested against each of the biotinylated peptides individually, in order to determine to which of the several peptides that were used as recognition units in the primary screen the phage are actually binding.

15 5.7. Use of Recognition Units from  
Known Amino Acid Sequences

In many cases it may not be necessary to screen a collection of substances, e.g., a peptide library, in order to obtain a recognition unit for a given functional domain. In the case of peptide recognition units, for example, it is sometimes possible to identify a recognition unit by inspection of known amino acid sequences. Stretches of these amino acid sequences that resemble known binding sequences for the functional domain can be synthesized and screened against a source of polypeptides in order to obtain a plurality of polypeptides comprising the given functional domain.

Prior to the disclosure of the present invention of methods of preparing recognition units having generic specificity, it would have been thought fruitless to pursue this approach. The expectation would have been that a recognition unit, chosen from published amino acid sequences as described above, would have been useful, at best, to identify a single protein containing a functional domain.

35

### 5.8. Isolation and Expression of Nucleic Acids Encoding Polypeptides Comprising a Functional Domain

In particular aspects, the invention provides amino acid sequences of polypeptides comprising functional domains, preferably human polypeptides, and fragments and derivatives thereof which comprise an antigenic determinant (i.e., can be recognized by an antibody) or which are functionally active, as well as nucleic acid sequences encoding the foregoing.

"Functionally active" material as used herein refers to that material displaying one or more functional activities, e.g., a biological activity, antigenicity (capable of binding to an antibody) immunogenicity, or comprising a functional domain that is capable of specific binding to a recognition unit.

In specific embodiments, the invention provides fragments of polypeptides comprising a functional domain consisting of at least 40 amino acids, or of at least 75 amino acids. Nucleic acids encoding the foregoing are provided. Functional fragments of at least 10 or 20 amino acids are also provided.

In other specific embodiments, the invention provides nucleotide sequences and subsequences encoding polypeptides comprising a functional domain, preferably human polypeptides, consisting of at least 25 nucleotides, at least 50 nucleotides, or at least 150 nucleotides. Nucleic acids encoding fragments of the polypeptides comprising a functional domain are provided, as well as nucleic acids complementary to and capable of hybridizing to such nucleic acids. In one embodiment, such a complementary sequence may be complementary to a cDNA sequence encoding a polypeptide comprising a functional domain of at least 25 nucleotides, or of at least 100 nucleotides. In a preferred aspect, the invention utilizes cDNA sequences encoding human polypeptides comprising a functional domain or a portion thereof.

Any eukaryotic cell can potentially serve as the nucleic acid source for the molecular cloning of polypeptides comprising a functional domain. The DNA may be obtained by standard procedures known in the art (e.g., a DNA "library") by cDNA cloning, or by the cloning of genomic DNA, or



fragments thereof, purified from the desired cell (see, for example Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, 2d. Ed., Cold Spring Harbor, New York; Glover, D.M. (ed.), 1985, DNA Cloning: A  
5 Practical Approach, MRL Press, Ltd., Oxford, U.K. Vol. I, II.) Clones derived from genomic DNA may contain regulatory and intron DNA regions in addition to coding regions; clones derived from cDNA will contain only exon sequences. Whatever the source, the gene encoding a polypeptide comprising a  
10 functional domain should be molecularly cloned into a suitable vector for propagation of the gene.

In the molecular cloning of the gene from genomic DNA, DNA fragments are generated, some of which will encode the desired gene. The DNA may be cleaved at specific sites  
15 using various restriction enzymes. Alternatively, one may use DNase in the presence of manganese to fragment the DNA, or the DNA can be physically sheared, as for example, by sonication. The linear DNA fragments can then be separated according to size by standard techniques, including but not limited to,  
20 agarose and polyacrylamide gel electrophoresis and column chromatography.

Once a gene encoding a particular polypeptide comprising a functional domain has been isolated from a first species, it is a routine matter to isolate the corresponding  
25 gene from another species. Identification of the specific DNA fragment from another species containing the desired gene may be accomplished in a number of ways. For example, if an amount of a portion of a gene or its specific RNA from the first species, or a fragment thereof e.g., the functional  
30 domain, is available and can be purified and labeled, the generated DNA fragments from another species may be screened by nucleic acid hybridization to the labeled probe (Benton, W. and Davis, R., 1977, Science 196, 180; Grunstein, M. And Hogness, D., 1975, Proc. Natl. Acad. Sci. U.S.A. 72, 3961).  
35 Those DNA fragments with substantial homology to the probe will hybridize. In a preferred embodiment, PCR using primers that hybridize to a known sequence of a gene of one species

can be used to amplify the homolog of such gene in a different species. The amplified fragment can then be isolated and inserted into an expression or cloning vector. It is also possible to identify the appropriate fragment by restriction  
5 enzyme digestion(s) and comparison of fragment sizes with those expected according to a known restriction map if such is available. Further selection can be carried out on the basis of the properties of the gene. Alternatively, the presence of the gene may be detected by assays based on the physical,  
10 chemical, or immunological properties of its expressed product. For example, cDNA clones, or DNA clones which hybrid-select the proper mRNAs, can be selected which produce a protein that, e.g., has similar or identical electrophoretic migration, isoelectric focusing behavior, proteolytic digestion  
15 maps, in vitro aggregation activity ("adhesiveness") or antigenic properties as known for the particular polypeptide comprising a functional domain from the first species. If an antibody to that particular polypeptide is available, corresponding polypeptide from another species may be  
20 identified by binding of labeled antibody to the putatively polypeptide synthesizing clones, in an ELISA (enzyme-linked immunosorbent assay)-type procedure.

Genes encoding polypeptides comprising a functional domain can also be identified by mRNA selection by nucleic  
25 acid hybridization followed by in vitro translation. In this procedure, fragments are used to isolate complementary mRNAs by hybridization. Such DNA fragments may represent available, purified DNA of genes encoding polypeptides comprising a functional domain of a first species. Immunoprecipitation  
30 analysis or functional assays (e.g., ability to bind to a recognition unit) of the in vitro translation products of the isolated mRNAs identifies the mRNA and, therefore, the complementary DNA fragments that contain the desired sequences. In addition, specific mRNAs may be selected by  
35 adsorption of polysomes isolated from cells to immobilized antibodies specifically directed against polypeptides comprising a functional domain. A radiolabelled cDNA of a

gene encoding a polypeptide comprising a functional domain can be synthesized using the selected mRNA (from the adsorbed polysomes) as a template. The radiolabelled mRNA or cDNA may then be used as a probe to identify the DNA fragments that

5 represent the gene encoding the polypeptide comprising a functional domain of another species from among other genomic DNA fragments. In a specific embodiment, human homologs of mouse genes are obtained by methods described above. In various embodiments, the human homolog is hybridizable to the

10 mouse homolog under conditions of low, moderate, or high stringency. By way of example and not limitation, procedures using such conditions of low stringency are as follows (see also Shilo and Weinberg, 1981, Proc. Natl. Acad. Sci. USA 78:6789-6792): Filters containing DNA are pretreated for 6 h

15 at 40°C in a solution containing 35% formamide, 5X SSC, 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.1% PVP, 0.1% Ficoll, 1% BSA, and 500 µg/ml denatured salmon sperm DNA. Hybridizations are carried out in the same solution with the following modifications: 0.02% PVP, 0.02% Ficoll, 0.2% BSA, 100 µg/ml

20 salmon sperm DNA, 10% (wt/vol) dextran sulfate, and 5-20 X 10<sup>6</sup> cpm <sup>32</sup>P-labeled probe is used. Filters are incubated in hybridization mixture for 18-20 h at 40°C, and then washed for 1.5 h at 55°C in a solution containing 2X SSC, 25 mM Tris-HCl (pH 7.4), 5 mM EDTA, and 0.1% SDS. The wash solution is

25 replaced with fresh solution and incubated an additional 1.5 h at 60°C. Filters are blotted dry and exposed for autoradiography. If necessary, filters are washed for a third time at 65-68°C and reexposed to film. Other conditions of low stringency which may be used are well known in the art

30 (e.g., as employed for cross-species hybridizations).

By way of example and not limitation, procedures using conditions of high stringency are as follows: Prehybridization of filters containing DNA is carried out for 8 h to overnight at 65°C in buffer composed of 6X SSC, 50 mM

35 Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA, and 500 µg/ml denatured salmon sperm DNA. Filters are hybridized for 48 h at 65°C in prehybridization mixture

containing 100 µg/ml denatured salmon sperm DNA and 5-20 X 10<sup>6</sup> cpm of <sup>32</sup>P-labeled probe. Washing of filters is done at 37°C for 1 h in a solution containing 2X SSC, 0.01% PVP, 0.01% Ficoll, and 0.01% BSA. This is followed by a wash in 0.1X SSC  
5 at 50°C for 45 min before autoradiography. Other conditions of high stringency which may be used are well known in the art.

The identified and isolated gene encoding a polypeptide comprising a functional domain can then be  
10 inserted into an appropriate cloning vector. A large number of vector-host systems known in the art may be used. Possible vectors include, but are not limited to, plasmids or modified viruses, but the vector system must be compatible with the host cell used. Such vectors include, but are not limited to,  
15 bacteriophages such as lambda derivatives, or plasmids such as PBR322 or pUC plasmid derivatives. The insertion into a cloning vector can, for example, be accomplished by ligating the DNA fragment into a cloning vector which has complementary cohesive termini. However, if the complementary restriction  
20 sites used to fragment the DNA are not present in the cloning vector, the ends of the DNA molecules may be enzymatically modified. Alternatively, any site desired may be produced by ligating nucleotide sequences (linkers) onto the DNA termini; these ligated linkers may comprise specific chemically  
25 synthesized oligonucleotides encoding restriction endonuclease recognition sequences. In an alternative method, the cleaved vector and gene may be modified by homopolymeric tailing. Recombinant molecules can be introduced into host cells via transformation, transfection, infection, electroporation,  
30 etc., so that many copies of the gene sequence are generated.

In an alternative method, the desired gene may be identified and isolated after insertion into a suitable cloning vector in a "shot gun" approach. Enrichment for the desired gene, for example, by size fractionization, can be  
35 done before insertion into the cloning vector.

In specific embodiments, transformation of host cells with recombinant DNA molecules that incorporate the

isolated gene, cDNA, or synthesized DNA sequence enables generation of multiple copies of the gene. Thus, the gene may be obtained in large quantities by growing transformants, isolating the recombinant DNA molecules from the transformants  
5 and, when necessary, retrieving the inserted gene from the isolated recombinant DNA.

The nucleic acid coding for a polypeptide comprising a functional domain of the invention can be inserted into an appropriate expression vector, i.e., a vector which contains  
10 the necessary elements for the transcription and translation of the inserted protein-coding sequence. The necessary transcriptional and translational signals can also be supplied by the native gene encoding the polypeptide and/or its flanking regions. A variety of host-vector systems may be  
15 utilized to express the protein-coding sequence. These include but are not limited to mammalian cell systems infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus); microorganisms such as yeast containing yeast vectors, or  
20 bacteria transformed with bacteriophage, DNA, plasmid DNA, or cosmid DNA. The expression elements of vectors vary in their strengths and specificities. Depending on the host-vector system utilized, any one of a number of suitable transcription and translation elements may be used.

25 Any of the methods previously described for the insertion of DNA fragments into a vector may be used to construct expression vectors containing a chimeric gene consisting of appropriate transcriptional/translational control signals and the protein coding sequences. These  
30 methods may include *in vitro* recombinant DNA and synthetic techniques and *in vivo* recombinants (genetic recombination). Expression of nucleic acid sequence encoding a protein or peptide fragment may be regulated by a second nucleic acid sequence so that the protein or peptide is expressed in a host  
35 transformed with the recombinant DNA molecule. For example, expression of a protein may be controlled by any promoter/enhancer element known in the art. Promoters which

may be used to control gene expression include, but are not limited to, the SV40 early promoter region (Benoist and Chambon, 1981, Nature 290, 304-310), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto, 5 et al., 1980, Cell 22, 787-797), the herpes thymidine kinase promoter (Wagner et al., 1981, Proc. Natl. Acad. Sci. U.S.A. 78, 1441-1445), the regulatory sequences of the metallothionein gene (Brinster et al., 1982, Nature 296, 39-42); prokaryotic expression vectors such as the  $\beta$ -lactamase 10 promoter (Villa-Kamaroff, et al., 1978, Proc. Natl. Acad. Sci. U.S.A. 75, 3727-3731), or the tac promoter (DeBoer, et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80, 21-25); see also "Useful proteins from recombinant bacteria" in Scientific American, 1980, 242, 74-94; plant expression vectors 15 comprising the nopaline synthetase promoter region (Herrera-Estrella et al., Nature 303, 209-213) or the cauliflower mosaic virus 35S RNA promoter (Gardner, et al., 1981, Nucl. Acids Res. 9, 2871), and the promoter of the photosynthetic enzyme ribulose biphosphate carboxylase (Herrera-Estrella et 20 al., 1984, Nature 310, 115-120); promoter elements from yeast or other fungi such as the Gal 4 promoter, the ADC (alcohol dehydrogenase) promoter, PGK (phosphoglycerol kinase) promoter, alkaline phosphatase promoter, and the following animal transcriptional control regions, which exhibit tissue 25 specificity and have been utilized in transgenic animals: elastase I gene control region which is active in pancreatic acinar cells (Swift et al., 1984, Cell 38, 639-646; Ornitz et al., 1986, Cold Spring Harbor Symp. Quant. Biol. 50, 399-409; MacDonald, 1987, Hepatology 7, 425-515); insulin gene control 30 region which is active in pancreatic beta cells (Hanahan, 1985, Nature 315, 115-122), immunoglobulin gene control region which is active in lymphoid cells (Grosschedl et al., 1984, Cell 38, 647-658; Adames et al., 1985, Nature 318, 533-538; Alexander et al., 1987, Mol. Cell. Biol. 7, 1436-1444), mouse 35 mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder et al., 1986, Cell 45, 485-495), albumin gene control region which is

active in liver (Pinkert et al., 1987, Genes and Devel. 1, 268-276), alpha-fetoprotein gene control region which is active in liver (Krumlauf et al., 1985, Mol. Cell. Biol. 5, 1639-1648; Hammer et al., 1987, Science 235, 53-58; alpha 1-  
5 antitrypsin gene control region which is active in the liver (Kelsey et al., 1987, Genes and Devel. 1, 161-171), beta-globin gene control region which is active in myeloid cells (Mogam et al., 1985, Nature 315, 338-340; Kollias et al., 1986, Cell 46, 89-94; myelin basic protein gene control region  
10 which is active in oligodendrocyte cells in the brain (Readhead et al., 1987, Cell 48, 703-712); myosin light chain-2 gene control region which is active in skeletal muscle (Sani, 1985, Nature 314, 283-286), and gonadotropic releasing hormone gene control region which is active in the  
15 hypothalamus (Mason et al., 1986, Science 234, 1372-1378).

Expression vectors containing inserts of genes encoding polypeptides comprising a functional domain can be identified by three general approaches: (a) nucleic acid hybridization, (b) presence or absence of "marker" gene  
20 functions, and (c) expression of inserted sequences. In the first approach, the presence of a foreign gene inserted in an expression vector can be detected by nucleic acid hybridization using probes comprising sequences that are homologous to the inserted gene. In the second approach, the  
25 recombinant vector/host system can be identified and selected based upon the presence or absence of certain "marker" gene functions (e.g., thymidine kinase activity, resistance to antibiotics, transformation phenotype, occlusion body formation in baculovirus, etc.) caused by the insertion of  
30 foreign genes in the vector. For example, if the gene encoding a polypeptide comprising a functional domain is inserted within the marker gene sequence of the vector, recombinants containing the gene can be identified by the absence of the marker gene function. In the third approach,  
35 recombinant expression vectors can be identified by assaying the foreign gene product expressed by the recombinant. Such assays can be based, for example, on the physical or

functional properties of the gene product in in vitro assay systems, e.g., ability to bind to recognition units.

Once a particular recombinant DNA molecule is identified and isolated, several methods known in the art may be used to propagate it. Once a suitable host system and growth conditions are established, recombinant expression vectors can be propagated and prepared in quantity. As previously explained, the expression vectors which can be used include, but are not limited to, the following vectors or their derivatives: human or animal viruses such as vaccinia virus or adenovirus; insect viruses such as baculovirus; yeast vectors; bacteriophage vectors (e.g., lambda), and plasmid and cosmid DNA vectors, to name but a few.

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Expression from certain promoters can be elevated in the presence of certain inducers; thus, expression of the protein may be controlled. Furthermore, different host cells have characteristic and specific mechanisms for the translational and post-translational processing and modification (e.g., glycosylation, cleavage) of proteins. Appropriate cell lines or host systems can be chosen to ensure the desired modification and processing of the foreign protein expressed. For example, expression in a bacterial system can be used to produce an unglycosylated core protein product. Expression in yeast will produce a glycosylated product. Expression in mammalian cells can be used to ensure "native" glycosylation of a heterologous protein. Furthermore, different vector/host expression systems may effect processing reactions such as proteolytic cleavages to different extents.

In other specific embodiments, polypeptides comprising a functional domain, or fragments, analogs, or derivatives thereof may be expressed as a fusion, or chimeric protein product (comprising the polypeptide, fragment, analog, or derivative joined via a peptide bond to a heterologous protein sequence (of a different protein)). Such a chimeric



product can be made by ligating the appropriate nucleic acid sequences encoding the desired amino acid sequences to each other by methods known in the art, in the proper reading frame, and expressing the chimeric product by methods commonly known in the art. Alternatively, such a chimeric product may be made by protein synthetic techniques, e.g., by use of a peptide synthesizer.

10           5.8.1       Identification and Purification of the Expressed Gene Product

          Once a recombinant which expresses the gene sequence encoding a polypeptide comprising a functional domain is identified, the gene product may be analyzed. This can be achieved by assays based on the physical or functional properties of the product, including radioactive labelling of the product followed by analysis by gel electrophoresis.

          Once the polypeptide comprising a functional domain is identified, it may be isolated and purified by standard methods including chromatography (e.g., ion exchange, affinity, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. The functional properties may be evaluated using any suitable assay, including, but not limited to, binding to a recognition unit.

25           5.9       Derivatives and Analogs of Polypeptides Comprising a Functional Domain

          The invention further provides derivatives (including but not limited to fragments) and analogs of polypeptides that are functionally active, e.g., comprising a functional domain. In a specific embodiment, the derivative or analog is functionally active, i.e., capable of exhibiting one or more functional activities associated with a full-length wild-type polypeptide, e.g., binding to a recognition unit. As one example, such derivatives or analogs may have the antigenicity of the full-length polypeptide.

In particular, derivatives can be made by altering gene sequences encoding polypeptides comprising a functional domain by substitutions, additions or deletions that provide for functionally equivalent molecules. Due to the degeneracy of nucleotide coding sequences, other DNA sequences which encode substantially the same amino acid sequence as a gene encoding a polypeptide comprising a functional domain may be used in the practice of the present invention. These include but are not limited to nucleotide sequences comprising all or portions of such genes which are altered by the substitution of different codons that encode a functionally equivalent amino acid residue within the sequence, thus producing a silent change. Likewise, the derivatives of the invention include, but are not limited to, those containing, as a primary amino acid sequence, all or part of the amino acid sequence of a polypeptide comprising a functional domain including altered sequences in which functionally equivalent amino acid residues are substituted for residues within the sequence resulting in a silent change. For example, one or more amino acid residues within the sequence can be substituted by another amino acid of a similar polarity which acts as a functional equivalent, resulting in a silent alteration. Substitutes for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs. For example, the nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid.

Derivatives or analogs of genes encoding polypeptides comprising a functional domain include but are not limited to those polypeptides which are substantially homologous to the genes or fragments thereof, or whose

encoding nucleic acid is capable of hybridizing to a nucleic acid sequence of the genes.

The derivatives and analogs of the invention can be produced by various methods known in the art. The  
5 manipulations which result in their production can occur at the gene or protein level. For example, the cloned gene sequence can be modified by any of numerous strategies known in the art (Maniatis, T., 1989, Molecular Cloning, A Laboratory Manual, 2d ed., Cold Spring Harbor Laboratory, Cold  
10 Spring Harbor, New York). The sequence can be cleaved at appropriate sites with restriction endonuclease(s), followed by further enzymatic modification if desired, isolated, and ligated in vitro. PCR primers can be constructed so as to introduce desired sequence changes during PCR amplification of  
15 a nucleic acid encoding the desired polypeptide. In the production of the gene encoding a derivative or analog, care should be taken to ensure that the modified gene remains within the same translational reading frame, uninterrupted by translational stop signals, in the gene region where the  
20 desired activity is encoded.

Additionally, the sequence of the genes encoding polypeptides comprising a functional domain can be mutated in vitro or in vivo, to create and/or destroy translation, initiation, and/or termination sequences, or to create  
25 variations in coding regions and/or form new restriction endonuclease sites or destroy preexisting ones, to facilitate further in vitro modification. Any technique for mutagenesis known in the art can be used, including but not limited to, in vitro site-directed mutagenesis (Hutchinson, C., et al., 1978,  
30 J. Biol. Chem 253:6551), use of TAB® linkers (Pharmacia), etc.

Manipulations of the sequence may also be made at the protein level. Included within the scope of the invention are protein fragments or other derivatives or analogs which are differentially modified during or after translation, e.g.,  
35 by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other

cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease,  $\text{NaBH}_4$ ;

5 acetylation, formylation, oxidation, reduction; metabolic synthesis in the presence of tunicamycin; etc.

In addition, analogs and derivatives can be chemically synthesized. For example, a peptide corresponding to a portion of a polypeptide comprising a functional domain  
10 can be synthesized by use of a peptide synthesizer.

Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the sequence. Non-classical amino acids include but are not limited to the D-isomers of the common amino  
15 acids,  $\alpha$ -amino isobutyric acid, 4-aminobutyric acid, hydroxyproline, sarcosine, citrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine,  $\beta$ -alanine, designer amino acids such as  $\beta$ -methyl amino acids,  $\text{C}\alpha$ -methyl amino acids, and  $\text{N}\alpha$ -methyl amino  
20 acids.

#### 5.10 Antibodies to Polypeptides Comprising a Functional Domain

---

According to one embodiment, the invention provides  
25 antibodies and fragments thereof containing the binding domain thereof, directed against polypeptides comprising a functional domain. Accordingly, polypeptides comprising a functional domain, fragments or analogs or derivatives thereof, in particular, may be used as immunogens to generate antibodies  
30 against such polypeptides, fragments or analogs or derivatives. Such antibodies can be polyclonal, monoclonal, chimeric, single chain, Fab fragments, or from an Fab expression library. In a specific embodiment, antibodies specific to the functional domain of a polypeptide comprising  
35 a functional domain may be prepared.

Various procedures known in the art may be used for the production of polyclonal antibodies. In a particular

embodiment, rabbit polyclonal antibodies to an epitope of a polypeptide comprising a functional domain, or a subsequence thereof, can be obtained. For the production of antibody, various host animals can be immunized by injection with the native polypeptide comprising a functional domain, or a synthetic version, or fragment thereof, including but not limited to rabbits, mice, rats, etc. Various adjuvants may be used to increase the immunological response, depending on the host species, and including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum.

For preparation of monoclonal antibodies, any technique which provides for the production of antibody molecules by continuous cell lines in culture may be used. For example, the hybridoma technique originally developed by Kohler and Milstein (1975, Nature 256, 495-497), as well as the trioma technique, the human B-cell hybridoma technique (Kozbor et al., 1983, Immunology Today 4, 72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al., 1985, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96).

Antibody fragments which contain the idiotype (binding domain) of the molecule can be generated by known techniques. For example, such fragments include but are not limited to: the  $F(ab')_2$  fragment which can be produced by pepsin digestion of the antibody molecule; the  $Fab'$  fragments which can be generated by reducing the disulfide bridges of the  $F(ab')_2$  fragment, and the  $Fab$  fragments which can be generated by treating the antibody molecule with papain and a reducing agent.

In the production of antibodies, screening for the desired antibody can be accomplished by techniques known in the art, e.g. ELISA (enzyme-linked immunosorbent assay).

## 6. EXAMPLES

### 6.1. Identification of Genes from cDNA Expression Libraries

5 A study was initiated to determine whether peptide recognition units could recognize functional domains that are the same as or similar to their target functional domain but that are contained in proteins other than the protein containing their target functional domain. Such "functional" screens, using recognition units of relatively small size, 10 were not previously known and were difficult to develop because of the low degree of sequence homology among functional domain-containing proteins. Thus, for example, an oligonucleotide probe could not be designed with any degree of confidence based on the low degree of homology of primary 15 sequences of SH3 domains.

Using SH3 domain-binding peptides from combinatorial peptide libraries as recognition units, we screened a series of mouse and human cDNA expression libraries. We found that 20 69 of the 74 clones isolated from the libraries encoded at least one SH3 domain. These clones represent more than 18 different SH3 domain-containing proteins, of which more than 10 have not been described previously.

The initial recognition unit chosen was a Src SH3 domain-binding peptide (termed pSrcCII) isolated from a phage- 25 displayed random peptide library (Sparks et al., 1994, J. Biol. Chem. 269:23853-23856). pSrcCII was (biotin-SGSGGILAPPVPPRNTR-NH<sub>2</sub>) (SEQ ID NO:1). pSrcCII was synthesized by standard Fmoc chemistry, purified by HPLC, and its 30 structure was confirmed by mass spectrometry and amino acid analysis. To form multivalent complexes, 50 pmol biotinylated pSrcCII peptide was incubated with 2 µg streptavidin-alkaline phosphatase (SA-AP) (for a biotin:biotin-binding site ratio of 1:1). Excess biotin-binding sites were blocked by addition of 35 500 pmol biotin. Alternatively, 31.2 µl of 1 mg/ml SA-AP could have been incubated with 15 µl of 0.1 mM biotinylated peptide for 30 min at 4 °C. Ten µl of 0.1 mM biotin would

then be added, and the solution incubated for an additional 15 min.

A  $\lambda$ EXlox mouse 16 day embryo cDNA expression library was obtained from Novagen (Madison, WI). The cDNA library was  
5 screened according to published protocols (Young and Davis, 1983, Proc. Natl. Acad. Sci. USA 80:1194-1198). The library was plated at an initial density of 30,000 plaques/100 mm petri plate as follows. A library aliquot was diluted 1:1000 in SM (100 mM NaCl, 8 mM MgSO<sub>4</sub>, 50 mM Tris HCl pH 7.5, 0.01%  
10 gelatin). Three  $\mu$ l of diluted phage were added to 1.5 ml each of SM, 10 mM CaCl<sub>2</sub>/MgCl<sub>2</sub>, and an overnight culture of BL21(DE3)pLyse E. coli cells. BL21 overnight cultures were grown in 2xYT medium (1.6% tryptone, 1% yeast extract, and 0.5% NaCl) supplemented with 10 mM MgSO<sub>4</sub>, 0.2 % maltose, and  
15 25 $\mu$ g/ml chloramphenicol. This mixture was incubated 20 min at 37°C, after which 300  $\mu$ l were plated on each of 14 2xYT agar plates in 3 ml 0.8% 2xYT top agarose containing 25  $\mu$ g/ml chloramphenicol. Plaques were allowed to form for 6 hours at 37°C, after which isopropyl- $\beta$ -D-thiogalactopyranoside (IPTG)-  
20 soaked filters were applied. After an additional eight hours' incubation at 37°C, the filters were marked, removed from the plates, and washed three times with phosphate buffered saline (PBS; 137 mM NaCl, 2.7 mM KCl, 4.3 mM Na<sub>2</sub>HPO<sub>4</sub>, 1.4 mM KH<sub>2</sub>PO<sub>4</sub>), 0.1% Triton X-100. The filters were blocked for 1 hour in  
25 PBS, 2% bovine serum albumin (blocking solution) and subsequently incubated overnight at 4°C with fresh blocking solution plus streptavidin-alkaline phosphatase (SA-AP) complexed peptide. Approximately 1  $\mu$ g SA-AP complexed with peptide in 1 ml blocking solution was used for each filter.  
30 The filters were then subjected to four 15 minute washes with PBS, 0.1% Triton X-100. Bound SA-AP-peptide complexes were detected by incubation with 44 ml nitroblue tetrazolium chloride (NBT, 75 mg/ml in 70% dimethylformamide) and 33 ml of 5-bromo-4-chloro-3-indoyl-phosphate-p-toluidine salt (BCIP 50  
35 mg/ml in dimethylformamide) in 10 ml of alkaline phosphatase buffer (0.1 M Tris-HCl, pH 9.4, 0.1 M NaCl, 50 mM MgCl<sub>2</sub>); the signals were robust, often evident within a few minutes.

Positive plaques were cored with a Pasteur pipet and placed in 1 ml SM with a drop of chloroform. Lambda phage particles are structurally resistant to chloroform, which serves as a bacteriocidal agent. These cores were allowed to diffuse into solution for at least 1 hr before subsequent platings. Phage from cores were plated in 100  $\mu$ l each of SM, 10 mM  $\text{CaCl}_2/\text{MgCl}_2$ , and an overnight culture of BL21 (DE3) pLySE cells. Phage were plated with the intention of reducing the number of plaque forming units (pfu)/plate by roughly a factor of 10 with each screen (i.e.,  $3 \times 10^4$  in the primary screen,  $3 \times 10^3$  in the secondary, and so on). This was accomplished by diluting cores 1:1000 and plating 1-10  $\mu$ l/plate. Four screens were generally required to obtain isolated plaques.

Plasmids were rescued from the  $\lambda$ EXlox phage by cre-mediated excision in BM25.8 E. coli cells. For each clone, 5  $\mu$ l of a 1:100 dilution of phage were added to a solution containing 100  $\mu$ l SM and 100  $\mu$ l of an overnight culture of BM25.8 cells (grown in 2xYT media supplemented with 10 mM  $\text{MgSO}_4$ , 0.2 % maltose, 34  $\mu$ g/ml chloramphenicol, and 50  $\mu$ g/ml kanamycin). After 30 minutes at 37 °C, 100  $\mu$ l of this solution were spread on an LB amp agarose plate and incubated overnight at 37 °C. A single colony from each plate was used to inoculate 3 ml of 2xYT/amp and incubated overnight. Plasmid DNA was purified from the overnight culture using Promega Wizard Miniprep DNA purification kits (Promega, Madison, WI), extracted with an equal volume of phenol/chloroform followed by chloroform alone, and ethanol precipitated. This plasmid DNA was used to transform chemical-competent DH5 $\alpha$  cells. Three colonies from each transformation were used to inoculate 3 ml cultures; DNA was purified as described above. Approximately, 1/20 of each individually purified DNA sample from transformed cells was digested with EcoR1 and HindIII and examined by electrophoresis on a 1% agarose gel to determine insert size and DNA quality. One DNA prep for each clone was either sequenced manually using the dideoxy method or by an automated technique that uses fluorescent dideoxynucleotide terminators.



The T7 gene 10 primer located approximately 40 bp upstream of the EcoR1 restriction site was used conveniently in both cases.

Approximately 100 of  $1 \times 10^6$  plaques in the primary  
5 screen of the  $\lambda$ EXlox 16 day mouse embryo cDNA expression library exhibited significant pSrcCII-binding activity. Figure 5 is representative of filters from primary and tertiary screens. Of the eighteen positive clones that were isolated and sequenced, all were found to encode proteins with  
10 SH3 domains, although several clones appeared to be siblings or to originate from the same mRNA. Thus, the pSrcCII screen resulted in the identification of cDNAs encoding nine distinct SH3 domain-containing proteins (see Figure 9). The sequences of these proteins were compared to the sequences in GenBank  
15 with the computer program BLAST. Three of these proteins corresponded to entries in GenBank. SH3P1 appears to be the murine homologue of p53bp2, a p53-binding protein, p53bp2 (Iwabuchi et al., 1994, Proc. Natl. Acad. Sci. USA 91:6098-6102); SH3P6 resembles human MLN50, a gene amplified in some  
20 breast carcinomas (Tomasetto et al., 1995, Genomics 28:367-376); and SH3P5 is Cortactin, a protein implicated in cytoskeletal organization (Wu and Parsons, 1993, J. Cell Biol. 120:1417-1426). Six of the clones did not match entries in GenBank, indicating that the present invention can be used to  
25 identify novel SH3 domain-containing proteins. Of these novel proteins, SH3P2 contains three ankyrin repeats and a proline-rich region flanking its SH3 domain; SH3P7 and SH3P9 contain sequences related to regions in the proteins drebrin (Ishikawa et al., 1994, J. Biol. Chem. 269:29928-29933) and amphiphysin  
30 (David et al., 1994, FEBS Lett. 351:73-79), respectively. Finally, the novel proteins SH3P4 and SH3P8, although not similar to any known proteins, are highly related (89% amino acid similarity) to one another.

The present invention can be used as part of an  
35 iterative process in which a recognition unit is used to identify proteins containing functional domains which are, in turn, used to derive additional recognition units for

subsequent screens. For example, to define the binding specificity of these newly cloned SH3 domains, they can be overexpressed as glutathione S-transferase (GST)-fusion proteins in bacteria, which, in turn, can be used to screen a  
5 random peptide library in order to obtain recognition units which, in turn, can be used to screen cDNA libraries in order to obtain still more novel proteins containing SH3 domains.

The recognition unit binding preferences of two of the SH3 domains isolated in the pSrcCII screen described above  
10 (p53bp2 and Cortactin) have been described (Sparks et al., 1996, Proc. Natl. Acad. Sci. USA 93:1540-1544. Each of these SH3 domains recognizes recognition unit motifs related to, yet distinct from, the pSrcCII sequence. We used a synthetic peptide (pCort) containing the Cortactin SH3 recognition unit  
15 motif to screen the mouse embryo cDNA expression library. pCort was (biotin-SGSGSRLTPQSKPPLPPKPSWVSR-NH<sub>2</sub>) (SEQ ID NO:2). pCort was prepared and complexed with SA-AP as above for pSrcCII. Screening of the mouse embryo library with pCort was done as above for pSrcCII.

20 Twenty six clones, of varying signal strength, were isolated and twenty-one were found to encode SH3 domain containing proteins. The pCort screen yielded genes corresponding to nine distinct SH3 domain-containing proteins (see Figure 9), four of which corresponded to entries in  
25 GenBank. SH3P5 and SH3P6 are Cortactin and MLN50, discussed above; SH3P10 matched SPY75/HS1, a protein involved in IgE signaling (Fukamachi et al., 1994, J. Immunol. 152:642-652); and SH3P11 is Crk, an SH2 domain and SH3 domain-containing adaptor molecule (Knudsen et al., 1994, J. Biol. Chem.  
30 269:32781-32787). The five novel transcripts encode SH3P7, SH3P8, and SH3P9, discussed above; SH3P13, an additional member of the SH3P4/SH3P8 family; and SH3P12, a protein with three SH3 domains and a region sharing significant sequence similarity with the peptide hormone sorbin (Vagen-Descroiz M.  
35 et al., 1991, Eur. J. Biochem. 201:53-50).

Interestingly, the output from the pCort screen only partially overlapped with that of the pSrcCII screen: four of

the nine SH3-containing proteins isolated with pCort were not identified with pSrcCII. In addition, SH3P9, the protein identified most frequently (50%) in the pSrcCII screen was isolated at a much lower frequency (7%) with the pCort probe. 5 Thus, different recognition units can be used to identify distinct sets of SH3 domains.

In addition to possessing at least one SH3 domain, a prominent characteristic of the proteins identified in the pSrcCII and pCort screens is the position of the SH3 domain 10 within the proteins: twelve of thirteen proteins possess SH3 domains near their C-termini. Although pSrcCII binds well to the Src SH3 domain (Figure 8), Src (whose SH3 domain occurs near the N-terminus) was not identified in the pSrcCII screen. We suspect the bias was a consequence of the fact that the 15 mouse embryo cDNA library was constructed using oligo-dT-primed cDNA. Alternatively, it may be that the mRNA used to prepare the library contained very little, or no, Src transcripts.

A variant of the pSrcCII peptide (T12SRC.1) was used 20 to probe a  $\lambda$ gt22a human prostate cancer cell line cDNA library primed with oligo-dT and a  $\lambda$ gt11 human bone marrow library primed with random and oligo-dT primers. T12SRC.1 was (biotin-GILAPPVPPRNTR-NH<sub>2</sub>) (SEQ ID NO:3). T12SRC.1 was used in the initial screens together with the peptide T12SRC.4. 25 T12SRC.4 was (biotin-VLKRPLPIPPVTR-NH<sub>2</sub>) (SEQ ID NO:4). The  $\lambda$ gt22a human prostate cancer cell line cDNA library was made from the LNCaP prostate cancer cell line by using standard methods, i.e., the Superscript Lambda system for cDNA synthesis and cloning (Bethesda Research Laboratories, 30 Gaithersburg, MD). The  $\lambda$ gt11 human bone marrow cDNA expression library was obtained from Clonetch (Palo Alto, CA). The human libraries were screened and positive clones isolated as described above for the mouse 16 day embryo cDNA library, except that cDNA inserts of the  $\lambda$ gt11 and  $\lambda$ gt22a phage were 35 amplified by PCR rather than being rescued by cre-mediated excision. Of the  $1.2 \times 10^7$   $\lambda$ cDNA clones screened from these libraries, 30 exhibited detectable pSrcCII-binding activity.

Analysis of the positive clones revealed that they each encoded at least one SH3 domain, and that they originated from a total of six different transcripts (Figure 9). Three of these encode proteins possessing non-C-terminal SH3 domains, 5 indicating that the present invention can be used to identify active domains regardless of their position within a protein. Of the six proteins identified, only three matched GenBank entries. SH3P15 and SH3P16 are Fyn (Kawakami et al., 1988, Proc. Natl. Acad. Sci. USA 85:3870-3874 and Lyn (Yamanashi et 10 al., 1987, Mol. Cell. Biol. 7:237-243), respectively, two Src-family members possessing SH3 domains with ligand preferences similar to that of the Src SH3 domain (Rickles, 1994, EMBO J. 13:5598-5604); and SH3P14 appears to be the human homologue of murine H74, a protein of unknown function. The three 15 remaining proteins did not match entries in GenBank and include the human homolog of SH3P9, described above, and SH3P17 and SH3P18, fragments of two related (85% amino acid similarity) adaptor-like proteins comprised of at least four and three SH3 domains, respectively.

20 Examination of the primary sequences of the SH3 domains identified in this work reveals several interesting features (see Figure 10). Positions important for ligand binding by the Src SH3 domain (Feng et al., 1994, Science 266:1241-1247; Lescure et al., 1992, J. Mol. Biol. 228:387-94) 25 and essential for SH3 function in Grb2/Sem5 are conserved (Clark et al., 1992, Nature 356:340-344). In addition, the two gaps in the sequence alignment shown in Figure 10 correspond to regions of length variation observed among previously characterized SH3 domains. Surprisingly, the SH3 30 domains identified in this work are not significantly more similar to one another than they are to other known SH3 domains, with the exception of the mouse and human forms of SH3P9 and SH3P14 which are 100% and 83% identical, respectively. This result indicates that SH3 domains can vary 35 widely in primary structure and still bind proline-rich peptide recognition units selectively.

6.1.1. Nucleotide and Corresponding Amino Acid  
Sequences of Genes Identified from cDNA  
Expression Libraries

The nucleotide sequences of SH3P1, SH3P2, SH3P3, SH3P4, SH3P5, SH3P6, SH3P7, SH3P8, SH3P9, SH3P10, SH3P11, SH3P12, SH3P13, and SH3P14, the mouse genes identified by screening the 16 day mouse embryo cDNA expression library with the peptides pSrcII and pCort, are shown in Figures 18, 20, 22, 24, 26, 28, 30, 32, 34, 38, 40, 42A and B, 44, and 46A and B, respectively. The corresponding amino acid sequences of the mouse genes SH3P1, SH3P2, SH3P3, SH3P4, SH3P5, SH3P6, SH3P7, SH3P8, SH3P9, SH3P10, SH3P11, SH3P12, SH3P13, and SH3P14 are shown in Figures 19, 21, 23, 25, 27, 29, 31, 33, 35, 39, 41, 43, 45, and 47, respectively.

The nucleotide sequences of SH3P9, SH3P14, SH3P17, and SH3P18, human genes identified by screening the human bone marrow and human prostate cancer cDNA expression libraries with the peptide T12SRC.1, are shown in Figures 36, 48, 50, and 52, respectively. The corresponding amino acid sequences of the human genes SH3P9, SH3P14, SH3P17, and SH3P18 are shown in Figures 37, 49, 51, and 53, respectively.

Two genes, SH3P9 and SH3P14, were isolated from both mouse and human libraries.

The sequences of SH3P15 and SH3P16 are not shown. SH3P15 is Lyn and SH3P16 is Fyn.

Figure 54 shows the nucleotide sequence of clone 55, a novel human gene identified and isolated from a human bone marrow cDNA library (described in Section 6.1) using as recognition units a mixture of T12SRC.4 and pCort (described in Section 6.1) and the methods described in Section 6.1.

Figure 55 shows the amino acid sequence of clone 55.

Figure 56 shows the nucleotide sequence of clone 56, a novel human gene identified and isolated from a human bone marrow cDNA library (described in Section 6.1) using as recognition units a mixture of T12SRC.4 and pCort (described in Section 6.1) and the methods described in Section 6.1.

Figure 57 shows the amino acid sequence of clone 56.

Figure 58A shows the nucleotide sequence from position 1-1720 and Figure 58B shows the nucleotide sequence from position 1720-2873 of clone 65, a novel human gene identified and isolated from a human bone marrow cDNA library 5 (described in Section 6.1) using as recognition units a mixture of P53BP2.Con and Nck1.Con3 and the methods described in Section 6.1. P53BP2.Con and Nck1.Con3 are peptides, the amino acid sequences of which are biotin-SFAAPARPPVPPRKSRPGG-NH<sub>2</sub> (SEQ ID NO:201) and biotin-SFSFPPLPPAPGG-NH<sub>2</sub> (SEQ ID 10 NO:202), respectively. The sequences of P53BP2.Con and Nck1.Con3 are consensus sequences of recognition units that bind to the SH3 domains of p53bp2 and Nck, respectively.

Figure 59 shows the amino acid sequence of clone 65.

Figure 60 shows the nucleotide sequence of clone 34, 15 a novel human gene identified and isolated from a human prostate cancer cDNA library (described in Section 6.1) using as recognition units a mixture of T12SRC.1 and T12SRC.4 (described in Section 6.1) and the methods described in Section 6.1.

20 Figures 61A and 61B show the amino acid sequence of clone 34.

Figure 62 shows the nucleotide sequence of clone 41, a novel human gene identified and isolated from a human bone marrow cDNA library (described in Section 6.1) using as 25 recognition units a mixture of PXXP.NCK.S1/4 and PXXP.ABL.G1/2M and the methods described in Section 6.1. PXXP.NCK.S1/4 and PXXP.ABL.G1/2M are peptides, the amino acid sequences of which are biotin-SRSLSEVSPKPPIRSVLSLR-NH<sub>2</sub> (SEQ ID NO:222) and biotin-SRPPRWSPPVPLPTSLDSR-NH<sub>2</sub> (SEQ ID NO:223), 30 respectively. PXXP.NCK.S1/4 and PXXP.ABL.G1/2M bind to the SH3 domains of Nck and Abl, respectively

Figures 63A and 63B show the amino acid sequence of clone 41.

Figure 64 shows the nucleotide sequence of clone 53, 35 a novel human gene identified and isolated from a human prostate cancer cDNA library (described in Section 6.1) using

as recognition units a mixture of PXXP.NCK.S1/4 and PXXP.ABL.G1/2M and the methods described in Section 6.1.

Figures 65A and 65B show the amino acid sequence of clone 53.

5           Figures 66A and 66B show the nucleotide and amino acid sequence of clone 5, a novel human gene identified and isolated from a HELA cell cDNA library using as recognition units a mixture of T12SRC.1 and T12SRC.4 (described in Section 6.1) and the methods described in Section 6.1.

10

#### 6.2. Use of Peptides Resembling SH3 Domain Binding Sequences as Recognition Units

---

We inspected a number of published amino acid sequences and identified proline-rich stretches of amino acids that resembled consensus SH3 domain binding sequences.  
15       Peptides comprising these proline-rich sequences were synthesized and tested by the methods of the present invention for their ability to specifically bind to the novel SH3 domains described in Sections 6.1 and 6.1.1. Purified SH3  
20       domain-containing clones were spotted on a lawn of Y1090 host cells, grown for an appropriate amount of time, and plaque filter lifts were screened with biotinylated peptides complexed with streptavidin-alkaline phosphatase as described in Section 6.1.

25       The results are shown in Figures 12 and 13. As can be seen, in many cases the synthesized peptides were able to bind to the novel SH3 domains. This indicates that those synthesized peptides could have been used to identify those novel SH3 domains from sources of polypeptides.

30

#### 6.3. Valency of Peptide Recognition Units Affects Specificity of Recognition Units

---

6.3.1       Preconjugation of Peptide Recognition Units with Streptavidin-Alkaline Phosphatase Increases Affinity of the Recognition Units for  
35       Targets

---

As a preliminary test of the effect of the valency of peptide recognition units on the ability of those

recognition units to be used as probes to detect SH3 domains, biotinylated peptides that had been previously shown to bind the SH3 domains of either Src or Abl were tested for their ability to bind their respective SH3 domain when either  
5 preconjugated with streptavidin-alkaline phosphatase (SA-AP) or not so preconjugated. GST-SrcSH3 and GST-AblSH3 fusion proteins (produced as described in Sparks et al., 1994, J. Biol. Chem. 269:23853-23856) were resolved by 10% SDS-PAGE and transferred to an Immobilon D nylon membranes (Millipore, New  
10 Bedford, MA). The membranes were incubated in blocking solution for 1 hr at 25 °C and then incubated overnight at 4 °C with either biotinylated Src SH3 domain or biotinylated Abl SH3 domain binding peptides in either multivalent (SA-AP) or monovalent format. The filters were washed three times (15  
15 min each wash) in PBS/T and incubated with NBT and BCIP for color development. See Section 6.1 for further details of the detection process.

The results are shown in Figure 14. In panels A, the biotinylated peptides were preconjugated with SA-AP and  
20 then allowed to bind to the immobilized SH3 domains. Preconjugation was as described in Section 6.1. In panels B, the peptides were first allowed to bind to the immobilized SH3 domains and then the bound peptides were detected by adding SA-AP. In both cases, color development was as in Section  
25 6.1. The sequences of the peptides used were: Biotin-SGSGGILAPPVPPRNTR (SEQ ID NO:1) for the Src specific peptide and Biotin-SGSGSRPPRWSPPPVPLPTSLDSR (SEQ ID NO:41) for the Abl specific peptide. The results shown in Figure 14 demonstrate that preconjugation with SA-AP dramatically increases the  
30 strength of the signal detected.

#### 6.3.2. Preconjugation of Peptide Recognition Units with Streptavidin-Alkaline Phosphatase Results in Recognition of a Variety of SH3 Domains

35 Two µg of each of a panel of GST-SH3 domain fusion proteins were transferred to Immobilon D nylon membranes (Millipore, New Bedford, MA) using a dot-blot apparatus.



Biotinylated Src, Abl, or Cortactin SH3 domain-binding peptides were preconjugated to SA-AP and incubated with the filter; an alkaline-phosphatase driven color reaction was used to detect peptide binding. The panel of immobilized proteins 5 was also reacted with a polyclonal anti-GST antibody (Pharmacia, Piscataway, NJ). Sequences of the Src, Abl, and Cortactin-binding peptides were Biotin-SGSGVLKRPLPIPPVTR (SEQ ID NO:42), Biotin-SGSGSRPPRWSPPPVPLPTSLDSR (SEQ ID NO:41), and Biotin-SGSGSRLGEFSKPPIQKPTWMSR (SEQ ID NO:43), respectively. 10 As can be seen from the results shown in Figure 15, the preconjugated biotinylated peptides recognized not only their original target SH3 domains, but related domains as well. The Src peptide recognized the SH3 domains of Yes and Cortactin as well as the SH3 domain of Src; the Abl peptide 15 recognized the Cortactin SH3 domain as well as the Abl SH3 domain; and the Cortactin peptide recognized Src, Yes, Abl, Crk, and the C terminal Grb2 SH3 domains as well as recognizing the Cortactin SH3 domain.

The above experiment was performed utilizing SH3 20 domains that had been immobilized on nylon membranes. The following demonstrates that preconjugation with streptavidin also permits peptide recognition units to recognize a variety of SH3 domains when those domains are immobilized in the wells of a microtiter plate.

25 Five different peptide recognition units (pAbl, pPLC, pCrk, pSrcCI, pSrcCII) were tested in either multivalent or monovalent format for their ability to bind to seven different SH3 domains (Src, Abl, PLC $\gamma$ , Crk, Cortactin, Grb2N, Grb2C) in an ELISA. The sequences of these peptides were as 30 follows: pAbl, SGSGSRPPRWSPPPVPLPTSLDSR (SEQ ID NO:41); pPLC, SGSGSMPPPVPPRPPGTLGG (SEQ ID NO:66); pCrk, SGSGNYVNALPPGPPLPAKNGG (SEQ ID NO:67); pSrcCI, SGSGVLKRPLPIPPVTR (SEQ ID NO:42); pSrcCII, SGSGGILAPPVPPRNTR (SEQ ID NO:1). These peptides were biotinylated as in Section 35 6.1.

The SH3 domains were produced as GST-SH3 fusion proteins as described in Sparks et al., 1994, J. Biol. Chem.

269:23853-23856. Their purity and concentration were confirmed by SDS-PAGE and Bradford protein assays, respectively. The GST-SH3 fusion proteins were immobilized in the wells of microtiter plates as follows: Two micrograms of each GST-SH3 fusion protein were incubated in wells of a flat bottom enzyme linked immunoabsorbent assay (ELISA) microtiter plate (Costar, Cambridge, MA) in 100 mM NaHCO<sub>3</sub> for 1 hr 25 °C. One volume of SuperBlock blocking buffer (Pierce Chemical Co., Rockford, IL) was added to each well and incubated for an additional 30 min. Plates were washed three times with PBS/0.1% Tween-20/0.1% bovine serum albumin (BSA). Immobilized proteins were detected with SH3 domain-binding peptides in multivalent or monovalent formats using streptavidin-horseradish peroxidase (SA-HRP; Sigma Chemical Co., St. Louis, MO). For complexation of the biotinylated peptides and SA-HRP, peptide and SA-HRP concentrations were as described for SA-AP complexation in Section 6.1, but all incubations and washes were in PBS/0.1% Tween-20/0.1% BSA. Plates were washed five times before colorimetric reaction and before the addition of SA-HRP (monovalent format). The amount of bound SA-HRP was evaluated with the addition of 100 µl horseradish peroxidase substrate [2',2'-Azino-Bis 3-Ethylbenzthiazoline-6-Sulfonic Acid (ABTS), 0.05 % hydrogen peroxide, 50 mM sodium citrate, pH 5.0]. After 5-30 minutes of reaction time, the optical densities (OD) of the microtiter plate wells were measured with a microtiter plate scanner (Molecular Devices, Sunnyvale, CA) set for 405 nm wavelength. The results are shown in Figure 8. From Figure 8 it can be seen that the tetravalent (complexed) peptides display both increased affinity and broadened specificity toward SH3 targets. Binding of complexed peptides was, however, still restricted to SH3 domains; the complexes bind to neither GST (Figure 8) nor other unrelated proteins (data not shown). Thus, precomplexation with SA-AP decreases the specificity of the peptide recognition units but does not make the peptides non-specific. Rather, the peptides, when precomplexed,

recognize a variety of SH3 domains in addition to their target domains.

5           6.3.3.       Preconjugation of Peptide Recognition Units  
                      with Streptavidin-Alkaline Phosphatase Results  
                      in Recognition of a Variety of Expressed cDNA  
                      Clones

          Lambda phage clones of genes containing a variety of  
SH3 domains were isolated from screens of a 16 day mouse  
embryo cDNA expression library (Novagen, Madison, WI). For a  
10 description of the isolation of these cDNA clones, see Section  
6.1. Phage particles corresponding to individual lambda phage  
cDNA recombinants were spotted onto 2xYT-1.5 % agar petri  
plates onto which had been poured 3 ml of 2xYT-0.8 % agarose  
with 100  $\mu$ l of a BL21(DE3)pLysE *E. coli* culture grown  
15 overnight. After a 6 hr incubation at 37 °C, expression of  
the cDNA segments was induced with IPTG-soaked nitrocellulose  
filters. After overnight incubation, the expressed proteins  
had been transferred to the filters and the filters were then  
incubated with either biotinylated SH3-domain binding peptides  
20 preconjugated to SA-AP or a monoclonal antibody recognizing  
the T7-Tag fusion peptide ( $\alpha$ T7.10Mab; Novagen, Madison, WI).  
This antibody was used as a positive control since it  
recognized an epitope expressed by all the clones (part of the  
 $\phi$ 10 leader sequence common to all  $\lambda$ EXlox recombinants).  
25 Sequences of pSrcI, pSrcII, Cortactin, and CaM (Calmodulin  
binding) peptides were Biotin-SGSGVLKRPLPIPPVTR (SEQ ID  
NO:42), Biotin-SGSGGILAPPVPPRNR (SEQ ID NO:1), Biotin-  
SGSGSRLGEFSKPPIPQKPTWMSR (SEQ ID NO:43), and Biotin-  
STVPRWIEDSLRGGAARAQTRLASAK (SEQ ID NO:44), respectively.

30           The results are shown in Figure 16. From Figure 16  
it can be seen that precomplexation with SA-AP decreases the  
specificity of the peptide recognition units but does not make  
the peptides non-specific; none of the peptides react in a  
significant fashion with two negative control sequences,  $\alpha$ -  
35 actinin and calmodulin (CaM). Rather, the peptides, when  
precomplexed, recognize a variety of SH3 domain-containing

cDNA clones in addition to clones containing their target domains.

#### 6.4. Characterization of cDNA clone-encoded proteins

##### 5 6.4.1. Production of cDNA clone-encoded proteins

Purified DNA from all positive cDNA clones (ca. 18-20 positive clones per recognition unit) was used to transform chemical-competent BL21 cells (Hanahan et al., 1983, J. Mol. Biol. 166:557-580, the complete disclosure of which is  
10 incorporated by reference herein).

Colonies that appeared after growth overnight at 37 °C on 2xYT agar plates containing 100 µg/ml ampicillin were used to inoculate 4 ml cultures of 2xYT/amp. After 7 hours of incubation at 37 °C with shaking, IPTG was added to each  
15 culture to a final concentration of 100 µM. After an additional 2 hours of incubation, 1 ml of each culture was collected and centrifuged to pellet the cells. Cell pellets were resuspended in 400 µl 1x SDS/DTT loading buffer and boiled at 100 °C for 5 min. The resulting cell lysates were  
20 subjected to Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) on an 8% acrylamide gel. Gels were either Coomassie stained or transferred to Immobilon D membrane (Millipore) and blotted (Towbin et al., 1979, Proc. Natl. Acad. Sci. 76:4350-4354).

25

#### 6.5. Materials Used in Sections 6.1, 6.2, 6.3.1, 6.3.2, 6.3.3, and 6.4.1

<b>Blocking Solution</b>		
	Hepes (pH 8)	20 mM
30	MgCl <sub>2</sub>	5 mM
	KCl	1 mM
	Dithiothreitol	5 mM
	Milk Powder	5% w/v
<b>2xYT media (1L)</b>		
	Bacto tryptone	16 g
35	Yeast Extract	10 g
	NaCl	5 g
<b>2xYT agar plates</b>		

2xYT + 15 g agar/L

2xYT top agarose (8%)

2xYT + 8 g agarose/L

SDS/DTT loading buffer

5 (10 mL of 5x solution)

.5 M Tris base	0.61 g
8.5% SDS	0.85 g
27.5% sucrose	2.75 g
100 mM DTT	0.154 g
.03% Bromophenol Blue	3.0 mg

- 10 **Overnight cell cultures:**  
Inoculate media with one isolated colony of appropriate cell type and incubate 37 °C O/N with shaking

BL21 (DE3) pLysE

2xYT media

maltose 0.2%

15 MgSO<sub>4</sub> 10 mM

Chloramphenicol 25 µg/mL

BM25.8

2xYT media

maltose 0.2%

MgSO<sub>4</sub> 10 mM

20 Chloramphenicol 34 µg/ml

Kanamycin 50 µg/ml

#### 6.6. Other Functional Domains and Recognition Units

- In a manner similar to that described above for SH3 domains, recognition units directed to other functional domains of interest can be chosen for use in the present method. For example, as recognition units for a study of GST functional domains, the following GST-binding peptides can be used to screen a plurality of polypeptides: Class I CWSEWDGNEC (SEQ ID NO:46), CGQWADDGYC (SEQ ID NO:47), CEOWDGYGAC (SEQ ID NO:48), CWPFWDGSTC (SEQ ID NO:49), CMIWPDGEEC (SEQ ID NO:50), CESOWDGYDC (SEQ ID NO:51), CQQWKEDGWC (SEQ ID NO:52), or CLYOWDGYEC (SEQ ID NO:53); Class II - CMGDNLGDDC (SEQ ID NO:54), CMGDSLGO SC (SEQ ID NO:55), CMDDDLGKGC (SEQ ID NO:56), CMGENLGWSC (SEQ ID NO:57), or CLGESLGWMC (SEQ ID NO:58).

Moreover, the following SH2-binding peptides can be used according to the methods of the present invention to

identify SH2 domain-containing polypeptides: GDGYEEISP (SEQ ID NO:59) (for Src family), GDGYDEPSP (SEQ ID NO:60) (for Nck), GDGYDHPSP (SEQ ID NO:61) (for Crk), GDGYVIPSP (SEQ ID NO:62) (PLC $\gamma$ N), GDGYQNYSP (SEQ ID NO:63) (for PLC $\gamma$ C), GDGYMAMSP (SEQ ID NO:64) (for p85PI3KN and p85PI3KC), or GDGQNYSP (SEQ ID NO:65) (for Grb2). See, Yang, Cell 72:767-778, the complete disclosure of which is incorporated by reference herein.

Further, polypeptides with a "PH" functional domain (analogous to the proteins Vav, Bcr, Mso, PLC $\delta$ , Atk, or Pleckstrin) can be identified using PH-binding peptides, such as those described by Mayer et al., Cell 73:629-630, the complete disclosure of which is incorporated by reference herein.

Other recognition units can be readily contemplated, including other synthetic, semisynthetic, or naturally derived molecules.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

Various publications are cited herein, the disclosures of which are incorporated by reference in their entireties.

30

35

## WHAT IS CLAIMED IS:

1. A method of identifying a polypeptide comprising a functional domain of interest comprising:

- (a) contacting a multivalent recognition unit  
5 complex with a plurality of polypeptides; and  
(b) identifying a polypeptide having a selective binding affinity for said recognition unit complex.

2. The method of claim 1 in which said plurality of  
10 polypeptides is from a polypeptide expression library.

3. The method of claim 1 in which said plurality of polypeptides is obtained from a virus.

15 4. The method of claim 2 in which said expression library is a cDNA expression library.

5. The method of claim 2 in which said expression library is a genomic DNA library.

20

6. The method of claim 2 in which said expression library is a recombinant bacteriophage library.

7. The method of claim 6 in which said recombinant  
25 bacteriophage library is a recombinant M13 library.

8. The method of claim 2 in which said expression library is a recombinant plasmid or cosmid library.

30 9. The method of claim 1 in which the recognition unit is a peptide.

10. The method of claim 1 in which said recognition unit is a peptide having less than about 140 amino acid residues.

35

11. The method of claim 1 in which said recognition unit is a peptide having less than about 100 amino acid residues.

12. The method of claim 1 in which said recognition unit is a peptide having less than about 70 amino acid residues.

13. The method of claim 1 in which said recognition unit 5 is a peptide having about 6 to 60 amino acid residues.

14. The method of claim 1 in which said recognition unit is a peptide having 20 to 50 amino acid residues.

10 15. The method of claim 1 in which the valency of the recognition unit in the complex is at least two.

16. The method of claim 9 in which the valency of the recognition unit in the complex is at least two.

15

17. The method of claim 1 in which the valency of the recognition unit in the complex is at least four.

18. The method of claim 9 in which the valency of the 20 recognition unit in the complex is at least four.

19. The method of claim 17 in which the recognition unit complex is a complex comprising (a) avidin or streptavidin, and (b) biotinylated recognition units.

25

20. The method of claim 18 in which the recognition unit complex is a complex comprising (a) avidin or streptavidin, and (b) the biotinylated peptides.

30 21. The method of claim 2 in which said identifying step comprises selecting a positive clone, which harbors a DNA construct encoding a polypeptide having a selective affinity for said recognition unit and which polypeptide includes the functional domain of interest or a functional equivalent 35 thereof.



22. The method of claim 21 which further comprises determining the coding sequence of said DNA construct.

23. The method of claim 22 which further comprises  
5 deducing an amino acid sequence from said coding sequence.

24. The method of claim 1 in which said contacting step comprises immobilizing said recognition unit complex on a solid support and bringing a solution containing said  
10 plurality of polypeptides in contact with said immobilized recognition unit complex.

25. The method of claim 1 in which said contacting step comprises separating said plurality of polypeptides and  
15 bringing a solution of said recognition unit complex in contact with said separated polypeptides.

26. The method of claim 1 in which said identifying step includes selecting a polypeptide, among said plurality of  
20 polypeptides, having a selective affinity for said recognition unit and determining the amino acid sequence of said polypeptide.

27. The method of claim 1 in which said plurality of  
25 polypeptides is immobilized on a solid support.

28. The method of claim 27 in which said contacting step comprises contacting said solid support with a solution containing said recognition unit complex.  
30

29. The method of claim 28 which further comprises washing away any unbound recognition unit complex.

30. The method of claim 29 which further comprises  
35 detecting any recognition unit complex that remains bound to said solid support.

31. The method of claim 1 in which said selective binding affinity is on the order of about 1 nM to about 1 mM.

32. The method of claim 1 in which said selective binding affinity is on the order of about 10 nM to about 100  $\mu$ M.

33. The method of claim 1 in which said selective binding affinity is on the order of about 100 nm to about 10  $\mu$ M.

34. The method of claim 1 in which said selective binding affinity is on the order of about 100 nm to about 1  $\mu$ M.

15

35. The method of claim 9 in which said peptide is chosen from a random peptide library.

36. A method of identifying a polypeptide comprising a functional domain of interest comprising:

(a) contacting a multivalent recognition unit complex, which complex comprises (i) avidin or streptavidin, and (ii) biotinylated recognition units, with a plurality of polypeptides from a cDNA expression library, in which the recognition unit is a peptide having in the range of 6 to 60 amino acid residues; and

(b) identifying a polypeptide having a selective binding affinity for said recognition unit complex.

37. The method of claim 4 or 36 in which the cDNA expression library is a human cDNA expression library.

38. The method of claim 36 in which the peptide is previously identified by a method comprising screening a random peptide library to identify a peptide having selective binding affinity for the functional domain of interest or a functional equivalent thereof.

39. The method of claim 36 in which the functional domain of interest is a domain selected from the group consisting of an SH1, SH2, SH3, PH, PTB, LIM, armadillo, Notch/ankyrin repeat, zinc finger, leucine zippers, and helix-  
5 turn-helix.

40. The method of claim 1 in which the functional domain of interest is a domain selected from the group consisting of an SH1, SH2, SH3, PH, PTB, LIM, armadillo,  
10 Notch/ankyrin repeat, zinc finger, leucine zipper, and helix-turn-helix.

41. The method of claim 1, 37, or 38 in which the functional domain of interest is an SH3 domain.  
15

42. A method of identifying a polypeptide comprising an SH3 domain of interest comprising:

(a) contacting a multivalent recognition unit complex, which complex comprises (i) avidin or streptavidin,  
20 and (ii) biotinylated recognition units, with a plurality of polypeptides from a cDNA expression library, in which the recognition unit is a peptide having in the range of 6 to 60 amino acid residues and which selectively binds an SH3 domain; and

25 (b) identifying a polypeptide having a selective binding affinity for said recognition unit complex.

43. The method of claim 1 in which the functional domain of interest comprises a catalytic site.  
30

44. The method of claim 43 in which said catalytic site corresponds to that found in glutathione S-transferase.

45. A method of identifying a polypeptide comprising a  
35 functional domain of interest or a functional equivalent thereof comprising:

(a) screening a random peptide library to identify a peptide that selectively binds a functional domain of interest; and

(b) screening a cDNA or genomic expression library  
5 with said peptide or a binding portion thereof to identify a polypeptide that selectively binds said peptide.

46. The method of claim 45 in which the screening step  
(b) is carried out by use of said peptide in a multivalent  
10 peptide complex.

47. The method of claim 46 in which the screening step  
(b) is carried out by use of said peptide in a complex  
comprising streptavidin and biotinylated peptide.  
15

48. The method of claim 46 in which the screening step  
(b) is carried out by use of said peptide in the form of  
multiple antigen peptides (MAP).

20 49. The method of claim 46 in which the screening step  
(b) is carried out by use of said peptide cross-linked to  
bovine serum albumin or keyhole limpet hemocyanin.

50. A method of identifying a polypeptide comprising a  
25 functional domain of interest or a functional equivalent  
thereof comprising:

(a) screening a random peptide library to identify a  
plurality of peptides that selectively bind a functional  
domain of interest;

30 (b) determining at least part of the amino acid  
sequences of said peptides;

(c) determining a consensus sequence based upon the  
determined amino acid sequences of said peptides; and

(d) screening a cDNA or genomic expression library  
35 with a peptide comprising the consensus sequence to identify a  
polypeptide that selectively binds said peptide.

51. The method of claim 50 in which the screening step (d) is carried out by use of said peptide in a multivalent peptide complex.

5 52. A method of identifying a polypeptide comprising a functional domain of interest or a functional equivalent thereof comprising:

(a) screening a random peptide library to identify a first peptide that selectively binds a functional domain of  
10 interest;

(b) determining at least part of the amino acid sequence of said first peptide;

(c) searching a database containing the amino acid sequences of a plurality of expressed natural proteins to  
15 identify a protein containing an amino acid sequence homologous to the amino acid sequence of said first peptide; and

(d) screening a cDNA or genomic expression library with a second peptide comprising the sequence of said protein  
20 that is homologous to the amino acid sequence of said first peptide.

53. An assay kit comprising in one or more containers:

(a) a purified polypeptide containing a functional  
25 domain of interest, in which the functional domain of is a domain selected from the group consisting of an SH1, SH2, SH3, PH, PTB, LIM, armadillo, Notch/ankyrin repeat, zinc finger, leucine zipper, and helix-turn-helix; and

(b) a purified recognition unit having a selective  
30 binding affinity for said functional domain in said polypeptide.

54. The assay kit of claim 53 in which said polypeptide comprises an amino acid sequence selected from the group  
35 consisting of SEQ ID NOs: 8, 10, 12, 18, 20, 22, 24, 30, 32, 38, 40, 190, 192, 194, 196, 198, 200, and 221.

55. The assay kit of claim 53 in which said polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:113-115, 118-121, 125-128, 133-139, 204-218, and 219.

5

56. The assay kit of claim 53 in which said recognition unit is a peptide.

57. The assay kit of claim 53 in which said polypeptide  
10 or recognition unit is labeled.

58. The assay kit of claim 57 in which said polypeptide or recognition unit is labeled with an enzyme.

15 59. The assay kit of claim 57 in which said polypeptide or recognition unit is labeled with an epitope.

60. The assay kit of claim 57 in which said polypeptide or recognition unit is labeled with a chromogen.

20

61. The assay kit of claim 57 in which said polypeptide or recognition unit is labeled with biotin.

62. The assay kit of claim 53 in which said polypeptide  
25 or recognition unit is immobilized on a solid support.

63. An assay kit comprising in containers:

(a) a plurality of purified polypeptides, each polypeptide in a separate container and each polypeptide  
30 containing a functional domain of interest in which the functional domain of interest is a domain selected from the group consisting of an SH1, SH2, SH3, PH, PTB, LIM, armadillo, Notch/ankyrin repeat, zinc finger, leucine zipper, and helix-turn-helix; and

35 (b) at least one recognition unit having a selective binding affinity for said functional domain in each of said plurality of polypeptides.

64. An assay kit comprising in one or more containers:

(a) a plurality of purified polypeptides, each polypeptide in a separate container and each polypeptide containing an SH3 domain; and

5 (b) at least one peptide having a selective affinity for the SH3 domain in each of said plurality of polypeptides.

65. A kit comprising a plurality of purified  
10 polypeptides comprising a functional domain of interest, each polypeptide in a separate container, and each polypeptide having a functional domain of a different sequence but capable of displaying the same binding specificity.

15 66. The kit of claim 65 in which the polypeptides have an amino acid sequence selected from the group consisting of: SEQ ID NO: 8, 10, 12, 18, 20, 22, 24, 30, 32, 38, 40, 190, 192, 194, 196, 198, 200, and 221.

20 67. The kit of claim 65 in which the functional domain is an SH3 domain.

68. The kit of claim 65 in which the functional domain is an SH3 domain from a polypeptide having an amino acid  
25 sequence selected from the group consisting of: SEQ ID NO: 8, 10, 12, 18, 20, 22, 24, 30, 32, 38, 40, 190, 192, 194, 196, 198, 200, and 221.

69. A method for screening a potential drug candidate  
30 comprising:

(a) allowing at least one polypeptide comprising a functional domain of interest to come into contact with at least one recognition unit having a selective affinity for said functional domain in said polypeptide, in the presence of  
35 an amount of a potential drug candidate, such that said polypeptide and said recognition unit are capable of interacting when brought into contact with one another in the

absence of said drug candidate, and in which the functional domain of interest is a domain selected from the group consisting of an SH1, SH2, SH3, PH, PTB, LIM, armadillo, Notch/ankyrin repeat, zinc finger, leucine zipper, and helix-  
5 turn-helix; and

(b) determining the effect, if any, of the presence of the amount of said drug candidate on the interaction of said polypeptide with said recognition unit.

10        70. The method of claim 69 in which the effect of the drug candidate upon multiple, different interacting polypeptide-recognition unit pairs is determined in which at least some of said polypeptides have a functional domain that differs in sequence but is capable of displaying the same  
15 binding specificity as the functional domain in another of said polypeptides.

71. The method of claim 69 in which at least one of said at least one polypeptide or recognition unit contains a  
20 consensus functional domain and consensus recognition unit, respectively.

72. The method of claim 69 in which the polypeptide is a polypeptide identified by the method of claim 1.  
25

73. The method of claim 69 in which the drug candidate is an inhibitor of the polypeptide-recognition unit interaction that is identified by detecting a decrease in the binding of polypeptide to recognition unit in the presence of  
30 such inhibitor.

74. A purified polypeptide comprising an SH3 domain, said SH3 domain having an amino acid sequence selected from the group consisting of: SEQ ID NOS:113-115, 118-121, 125-128,  
35 133-139, 204-218, and 219.



75. A purified polypeptide comprising an SH3 domain, said polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 10, 12, 18, 20, 22, 24, 30, 32, 38, 40, 190, 192, 194, 196, 198, 200, and 221.

5

76. A purified DNA encoding an SH3 domain, said DNA having a sequence selected from the group consisting of SEQ ID NOs: 7, 9, 11, 17, 19, 21, 23, 29, 31, 37, 39, 189, 191, 193, 195, 197, 199, and 220.

10

77. A purified DNA encoding a polypeptide comprising an amino acid sequence selected from the group consisting of: SEQ ID NOs: 8, 10, 12, 18, 20, 22, 24, 30, 32, 38, 40, 190, 192, 194, 196, 198, 200, and 221.

15

78. A purified DNA encoding a polypeptide comprising an amino acid sequence selected from the group consisting of: SEQ ID NOs: 113-115, 118-121, 125-128, 133-139, 204-218, and 219.

20

79. A purified molecule comprising an SH3 domain of a polypeptide having an amino acid sequence selected from the group consisting of: SEQ ID NO: 8, 10, 12, 18, 20, 22, 24, 30, 32, 38, 40, 190, 192, 194, 196, 198, 200, and 221.

25

80. A fusion protein comprising (a) an amino acid sequence comprising an SH3 domain of a polypeptide having the amino acid sequence of SEQ ID NO: 8, 10, 12, 18, 20, 22, 24, 30, 32, 38, 40, 190, 192, 194, 196, 198, 200, or 221 joined via a peptide bond to (b) an amino acid sequence of at least  
30 six amino acids from a different polypeptide.

81. A purified DNA encoding the fusion protein of claim  
80.

35

82. A nucleic acid vector comprising the DNA of claim  
81.

83. A nucleic acid vector comprising the DNA of claim 76.

84. A nucleic acid vector comprising the DNA of claim 5 78.

85. A recombinant cell containing the nucleic acid vector of claim 82, 83, or 84.

10 86. A purified nucleic acid hybridizable to a nucleic acid having a sequence selected from the group consisting of: SEQ ID NOS: 7, 9, 11, 17, 19, 21, 23, 29, 31, 37, 39, 189, 191, 193, 195, 197, 199, and 220.

15 87. A method of producing the fusion protein of claim 80 comprising culturing a recombinant cell containing a nucleic acid vector encoding said fusion protein such that said fusion protein is expressed, and recovering the expressed fusion protein.

20 88. A method of producing the polypeptide of claim 74 comprising culturing a recombinant cell containing a nucleic acid vector encoding said polypeptide such that said polypeptide is expressed, and recovering the expressed 25 polypeptide.

89. The method of claim 69 in which said polypeptide is a polypeptide containing an SH3 domain produced by a method comprising:

- 30 (i) screening a peptide library with an SH3 domain to obtain one or more peptides that bind the SH3 domain;  
(ii) using one of the peptides from step (i) to screen a source of polypeptides to identify one or more polypeptides containing an SH3 domain;  
35 (iii) determining the amino acid sequence of the polypeptides identified in step (ii); and

(iv) producing the one or more novel polypeptides containing an SH3 domain.

90. The method of claim 69 in which said polypeptide is  
5 a polypeptide containing an SH3 domain produced by a method comprising:

(i) screening a peptide library with an SH3 domain to obtain a plurality of peptides that bind the SH3 domain;

(ii) determining a consensus sequence for the  
10 peptides obtained in step (i);

(iii) producing a peptide comprising the consensus sequence;

(iv) using the peptide comprising the consensus sequence to screen a source of polypeptides to identify one or  
15 more polypeptides containing an SH3 domain;

(v) determining the amino acid sequence of the polypeptides identified in step (iv); and

(vi) producing the one or more polypeptides containing an SH3 domain.

20

91. A method of determining the potential pharmacological activities of a molecule comprising:

(a) contacting the molecule with a compound comprising a functional domain under conditions conducive to  
25 binding;

(b) detecting or measuring any specific binding that occurs; and

(c) repeating steps (a) and (b) with a plurality of different compounds, each compound comprising a functional  
30 domain of different sequence but capable of displaying the same binding specificity.

92. The method of claim 91 in which the functional domain is an SH3 domain.

35

93. The method of claim 92 in which the compounds comprise the SH3 domains of Src, Abl, Cortactin, Phospholipase

Cy, Nck, Crk, p53bp2, Amphiphysin, Grb2, RasGap, or Phosphatidylinositol 3' kinase.

94. A method of identifying a compound that affects the binding of a molecule comprising a functional domain to a recognition unit that selectively binds to the functional domain comprising:

(a) contacting the molecule comprising the functional domain and the recognition unit under conditions conducive to binding in the presence of a candidate compound and measuring the amount of binding between the molecule and the recognition unit and in which the functional domain of interest is a domain selected from the group consisting of an SH1, SH2, SH3, PH, PTB, LIM, armadillo, Notch/ankyrin repeat, zinc finger, leucine zipper, and helix-turn-helix;

(b) comparing the amount of binding in step (a) with the amount of binding known or determined to occur between the molecule and the recognition unit in the absence of the candidate compound, where a difference in the amount of binding between step (a) and the amount of binding known or determined to occur between the molecule and the recognition unit in the absence of the candidate compound indicates that the candidate compound is a compound that affects the binding of the molecule comprising a functional domain and the recognition unit.

95. The method of claim 94 in which the functional domain is an SH3 domain.

96. The method of claim 20 in which the recognition unit complex is a complex comprising (a) streptavidin conjugated to alkaline phosphatase; and (b) the biotinylated peptides.

97. A method of identifying a polypeptide comprising a functional domain of interest comprising:

(a) contacting a recognition unit that is a peptide having 140 amino acids or fewer with a plurality of polypeptides; and

(b) identifying a polypeptide having a selective binding affinity for said recognition unit complex.

98. An antibody to a polypeptide comprising an amino acid sequence selected from the group consisting of: SEQ ID NOS:113-115, 118-121, 125-128, 133-139, 204-218, and 219.

10

99. An antibody to a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 8, 10, 12, 18, 20, 22, 24, 30, 32, 38, 40, 190, 192, 194, 196, 198, 200, and 221.

15

100. The purified nucleic acid of claim 86 that is a human nucleic acid encoding a polypeptide containing a functional domain.

101. A purified protein encoded by a first nucleic acid comprising a human cDNA or genomic sequence hybridizable to a second nucleic acid having a sequence selected from the group consisting of: SEQ ID NOS:7, 9, 11, 17, 19, 21, 29, and 31.

102. The assay kit of claim 53 in which said polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:6, 14, 16, 26, 28, 34, 36, 112, 116, 117, 122-124, 129-132, and 140.

30

35

1/61

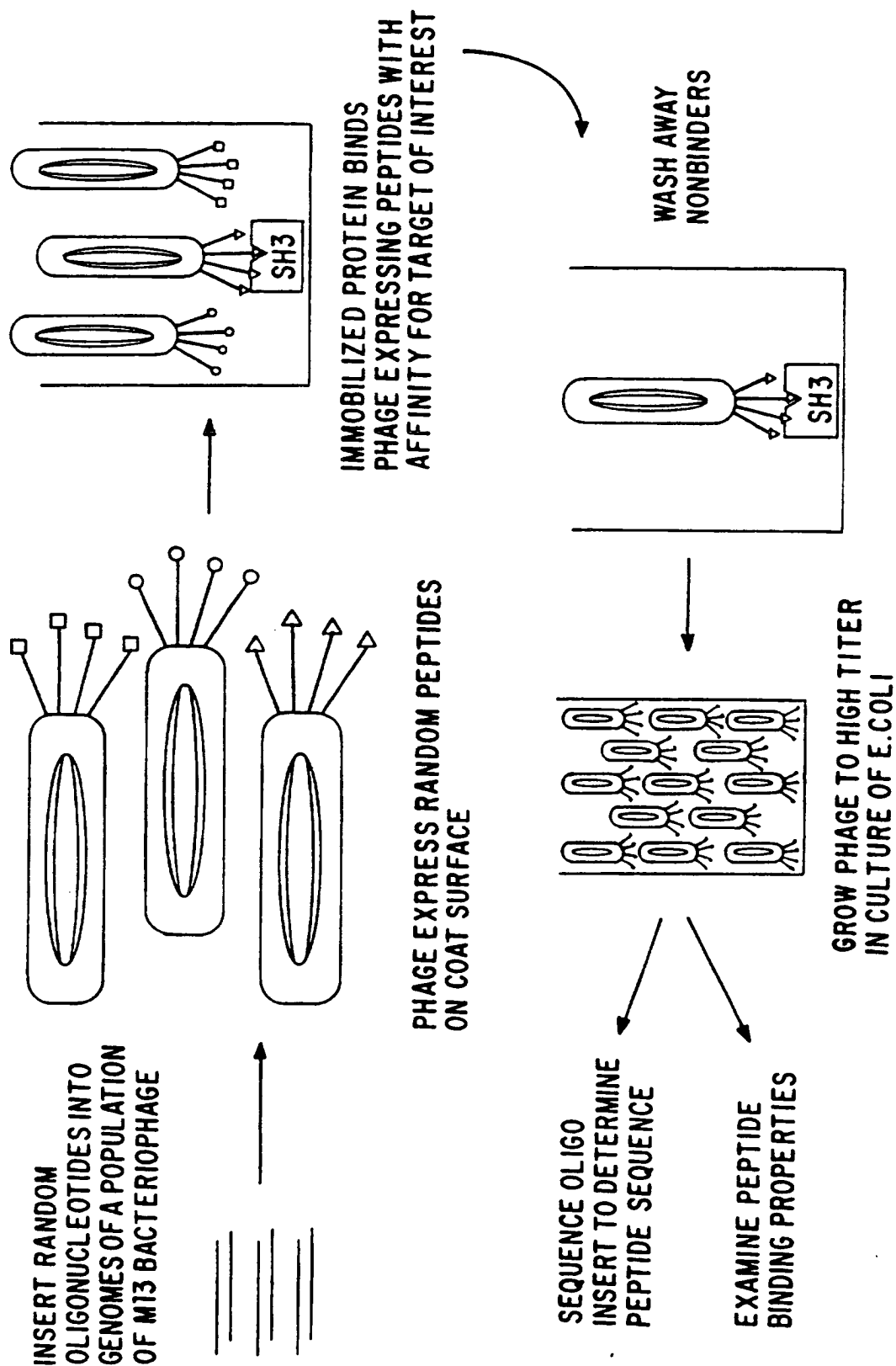


FIG. 1

SUBSTITUTE SHEET (RULE 26)

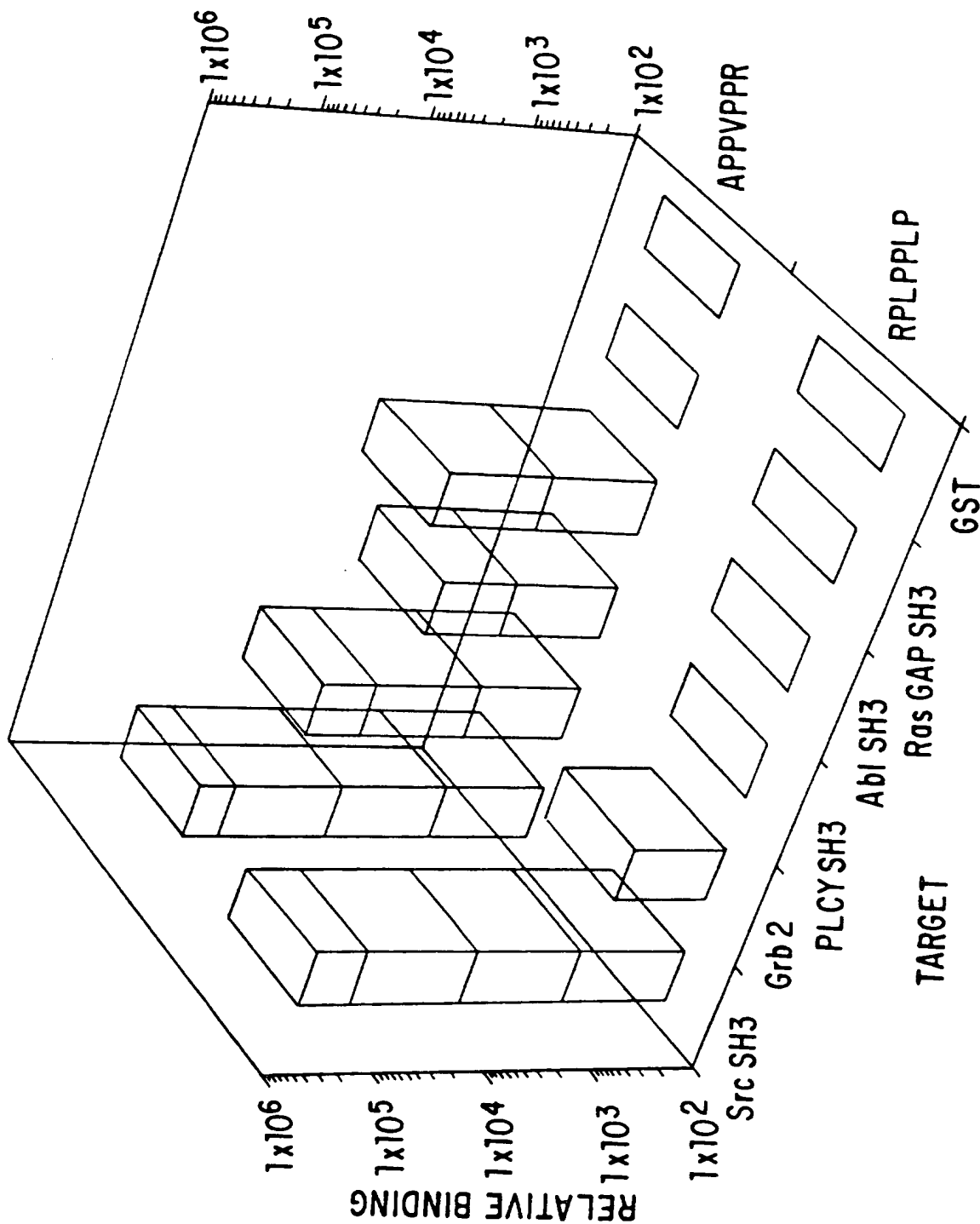


FIG. 2

SUBSTITUTE SHEET (RULE 26)

3/61

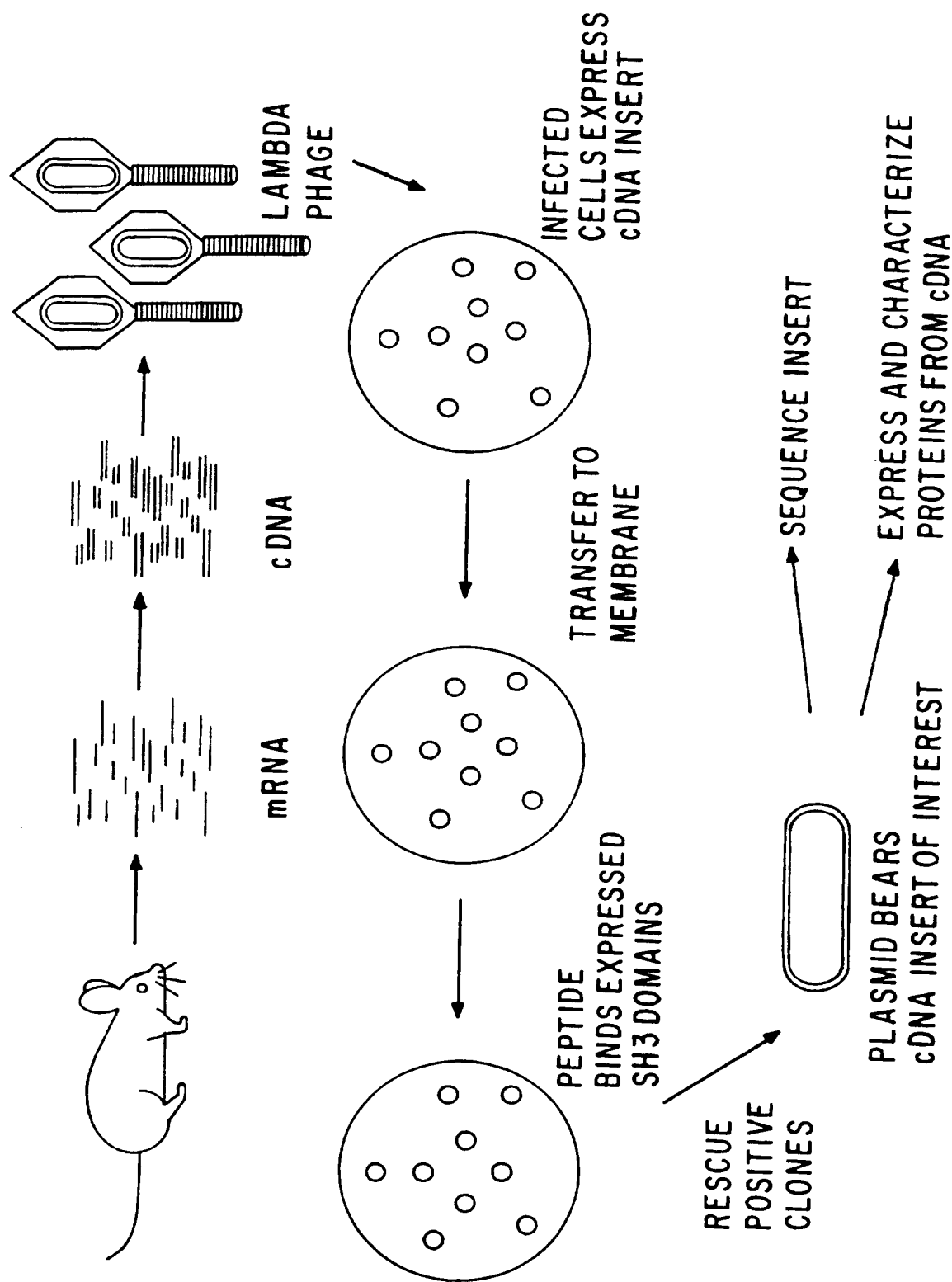


FIG. 3

SUBSTITUTE SHEET (RULE 26)



4/61

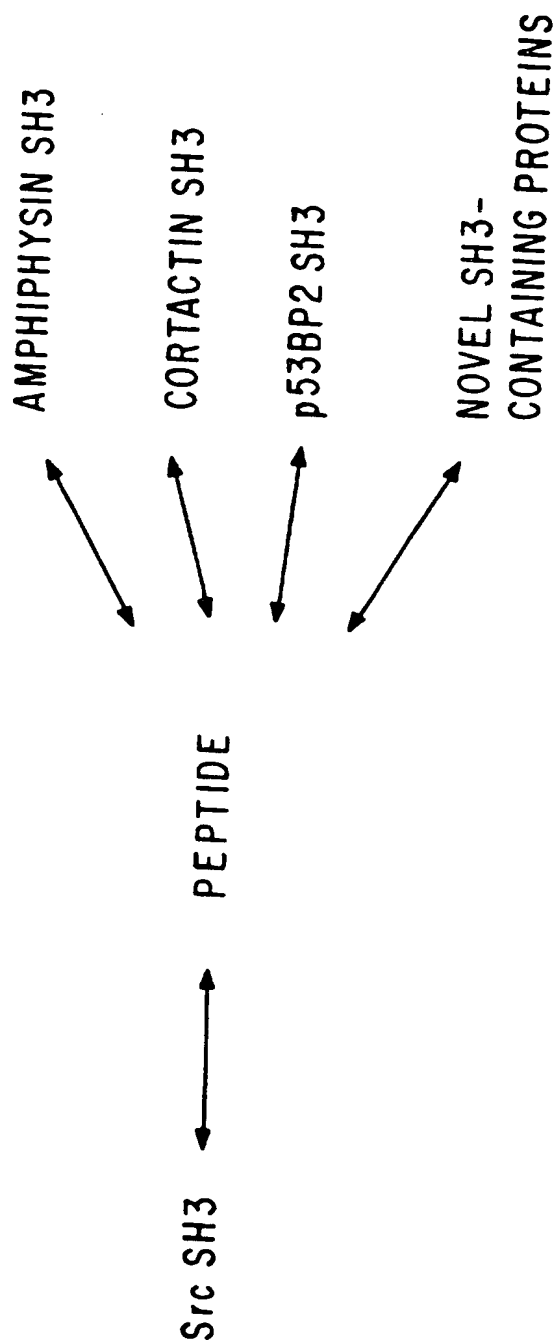


FIG. 4

SUBSTITUTE SHEET (RULE 26)

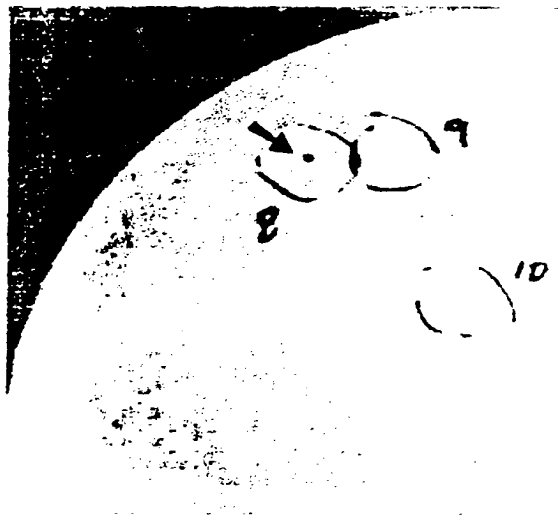


FIG. 5A



FIG. 5B

SUBSTITUTE SHEET (RULE 26)

6/61

Sc_Fus1_Sh3:	TVI QDYE PRLTDE.....	IRISLG EKVIL...A .....	THTD	CLVEKNTRK
Sc_Bob1_Sh3:	RAL FDYD KTKDCGFLSQ .....	ALSRFG DVLHVIDA.....	GDEE	QAR RVHSDSE
Hs_Mpp1_Sh3:	RAQ FDYD PKKDNLPCK .....	EAGLKATG DIIQINK.....	DDSN	QGR ...VEGSS
Hs_Zo1_Sh3:	RTH PHYE KESPY.....	GLSFNG EVFRAVDTL...NGKLS		AI RIGKNHKE
Hs_Ncf1_Csh3:	VAI KAYT AV.EGD..E.	VSLLEG EAVEVIHK.....	LLDG	V IRKDDVTGY
Hs_Rasgap_Sh3:	RAI LPYT KVPDTE..E.	ISFLKG DMFIVHNE.....	LED	.. MWVTNLR
Sc_Sla_Nsh3:	RAV YAVE ..PQTP..EE	LAIQED DLYLLQKSD.....	IDD	TVKKR VIGSD
Sc_Bem1_Nsh3:	KAK YSYQ ..AQTS..KE	LSFMKG EFFYV..SGD.....	EKD	KASNP STGKE
Hs_Ncf2_Nsh3:	AHR VLFG FVPETK..EE	LQMPG NIVFVL..KK .....	GND	ATVMFNG.QK
Sc_Sla_Csh3:	RGI VQYD FMAESQ..DE	LTIKSG DKVYILDDK .....	SKD	MCQLVDS.GK
Hs_Grb2_Csh3:	QAL FDPD ..POED..GE	LGFRG DFHVM..N S.....	DPN	KG.A.CH.GQ
Hs_Nck_Csh3:	QAL YPFS ..SSND..EE	LNFEKG DVMVIEKPE N.....	DPE	KCRK.IN.GM
Mm_Tec_Sh3:	VAM YDFG ..ATEA..HD	RLRERG QEYIIEKN.....	DLH	RARD.KN.GQ
Hs_Atk_Sh3:	VAL YDYN ..PMNA..ND	LQLRKG DEYFILEES.....	NLP	RARD.KY.GW
Hs_Ab1_Sh3:	VAL YDFV ..ASGD..NT	LSITKG EKLRVLGYN.....	HNGE	EAQT.KN.GQ
Hs_Src_Sh3:	VAL YDYE ..SRTE..TD	LSFKKG ERLQIVNNT.....	EGD	LAHSLT.GQ
Hs_Fgr_Sh3:	IAL YDYE ..ARTE..DD	LTFTKG EKFHILNNT.....	EGD	EARSLS.GK
Hs_Fyn_Sh3:	VAL YDYE ..ARTE..DD	LSFHKG EKFOILNSS.....	EGD	EARSLT.TE
Hs-Yes_Sh3:	VAL YDYE ..ARTT..ED	LSFKKG ERFQIINNT.....	EGD	EARSIA.TGK
Mm_Fgr_Sh3:	VAL YDYE ..ARTG..DD	LTFTKG EKFHILNNT.....	EYD	EARSLS.GH
Hv_Stk_Sh3:	VAL YDYE ..ARIS..ED	LSFKKG ERLQIINTA.....	DGD	YARSLT.NS
Hs_Hck_Sh3:	VAL YDYE ..AIHH..ED	LSFQKG DQMVVEES.....	GE	KARSLAT.RK
Hs_Lyn_Sh3:	VAL YPYD ..GIHP..DD	LSFKKG EKMKVLEEH.....	GE	KAKSLT.KK
Mm_Blk_Sh3:	VAL PDYA ..AVND..RD	LQVLKG EKQVLRST.....	GD	LARSLV.GR
Hs_Lck_Sh3:	IAL HSYE ..PSHD..GD	LGFEKG EQLRILEQS.....	GE	KAQSLT.TGQ
Hs_Nck_Nsh3:	VAK FDYV ..AQQE..QE	LDIKKN ERLWLLDDSK SW.....		VRNSM...NK
Sc_Sla_Msh3:	RAI YDYE QVQNAD..EE	LTfHEN DVFDVFKKD.....	DAD	LVKSTVS.NE

FIG. 6A

SUBSTITUTE SHEET (RULE 26)

7/61

Hs_Hs1_Sh3:	VAL YDQ	..GEGS..DE	....LSFDPD DVITDIEMV.	.....DEG	RG..RCH.GH
Sc_Abp1_Sh3:	TAE YDQ	..AAED..NE	....LTFVEN DKINIEFV.	.....DDD	LGELEKD.GS
Hs_Nck_Msh3:	YVK FNYN	..AERE..DE	....LSLKG TKVIMEKC.	.....SDG	RG..SYN.GQ
Hs_Vav_Sh3:	KAR YDFC	..ARDR..SE	....LSLKEG DIIKILNK.	.....GQQ	WRGEIY..GR
Hs_Grb2_Nsh3:	IAK YDFK	..ATAD..DE	....LSFKRG DILKVLNEE.	.....CDQ	YKAELN..GK
Hs_P1cg2_Sh3:	KAL YDYK	..AKRS..DE	....LSFCRG ALIHNSKE	.....PG	WKGDYGT.RI
Hs_P1cg1_Sh3:	KAL FDYK	..AQRE..DE	....LTFIKS AIIQNVEKQ.	.....EG	WRGDYGG.KK
Ac_Mys1b_Sh3:	KAL YDYD	..AQTG..DE	....LTFKEG DTIIVHQD.	.....PA	WEGELN..GK
Ac_Mys1c_Sh3:	RAL YDFA	..AENP..DE	....LTFNEG AVTVINKS.	.....NP	WEGELN..GQ
Dd_Mys1b_Sh3:	KAL YDYD	..ASST..DE	....LSFKEG DIIFIVQKD.	.....NG	TQGELKS.GQ
Hs_Ncf2_Csh3:	EAL FSYE	..ATQP..ED	....LEFQEG DIIILVLSKV.	.....NE	LEGECK..GK
Hs_Ncf1_Nsh3:	RAI ADYE	..KTSG..SE	....MALSTG DWVEVWEKS.	.....ESG	FCQM..K.AK
Hs_Spectrin_Sh3:	MAL VDFQ	..ARSP..RE	....VTMKG DVLTLSSI.	.....NKD	KVEA..A.DH
Sc_Bem1_Csh3:	YAI VLYD	FKAeka..DE	....LTTYVG ENLFICAH..	.....HNCE	IAKPIGRLLG
Sc_Cde25_Sh3:	VAA YDFN	YPIKkd..SS	..SQLLSVQQG ETIYILNKNS	SG	...DGL VIDD
Sp_Ste_Sh3:	MR FQTT	AISDYENSSN	..PSFLKFSAG DTIIIVIEVLE	D	CDG.....
Hs_Pf3ka_Sh3:	RAL VDYK	KEREEDIDLH	LGDI LTVNKG SLVALGFSDG	QEARPEEI	LNGYNETTGE

FIG. 6B

SUBSTITUTE SHEET (RULE 26)

8/61

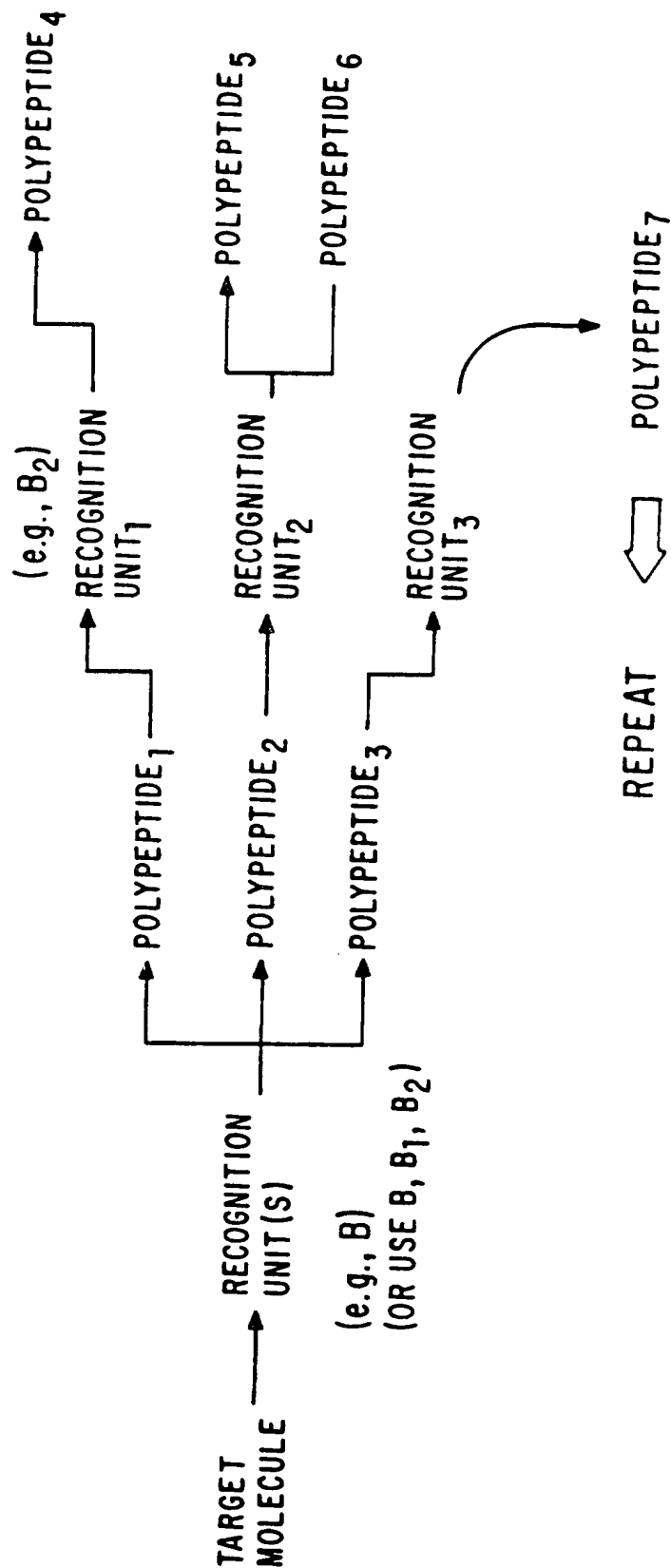


FIG. 7B

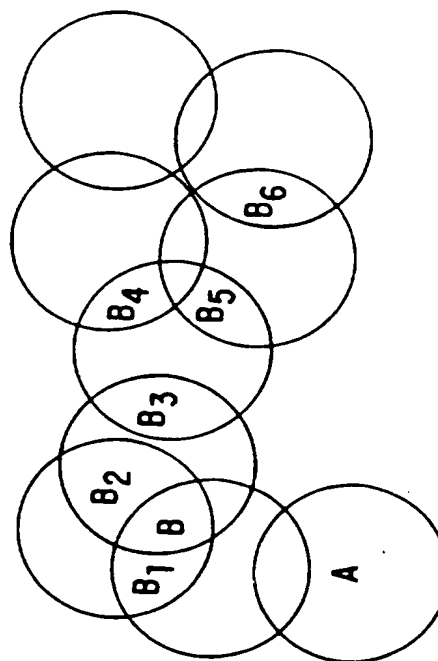


FIG. 7A

SUBSTITUTE SHEET (RULE 26)

9/61

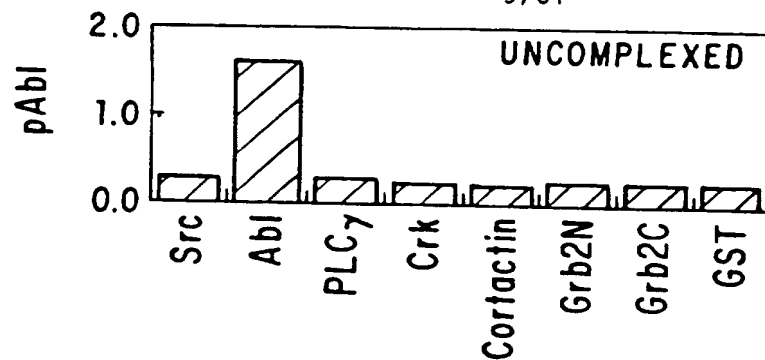


FIG. 8A

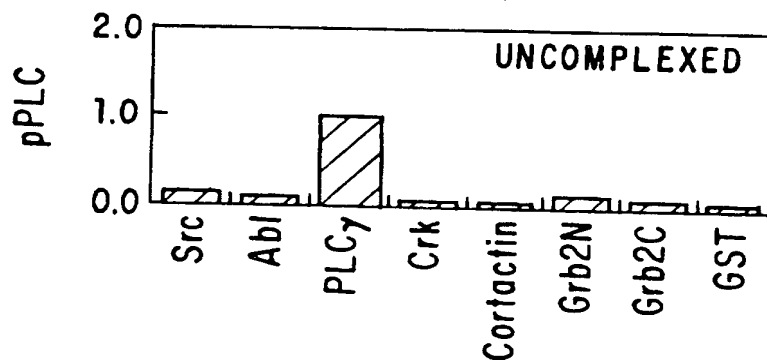


FIG. 8B

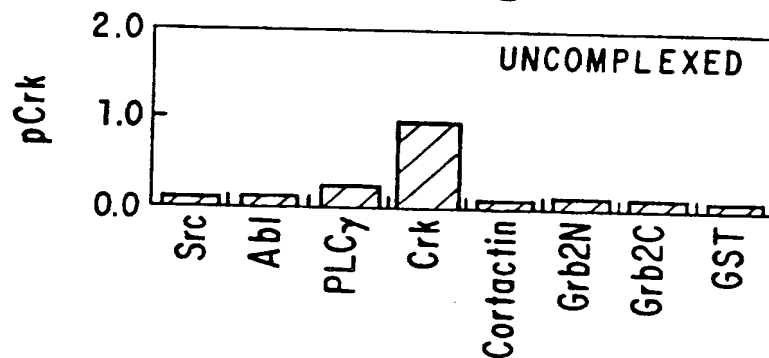


FIG. 8C

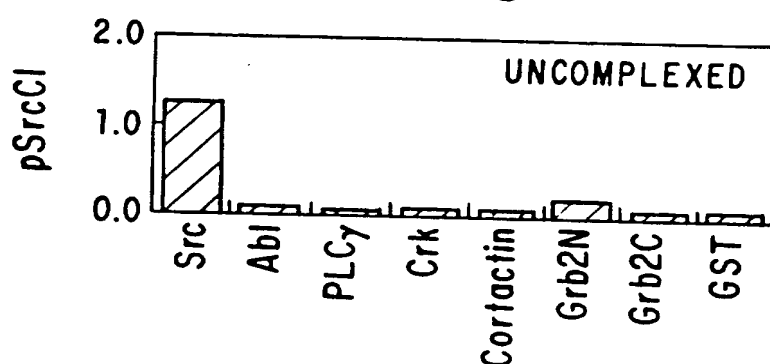


FIG. 8D

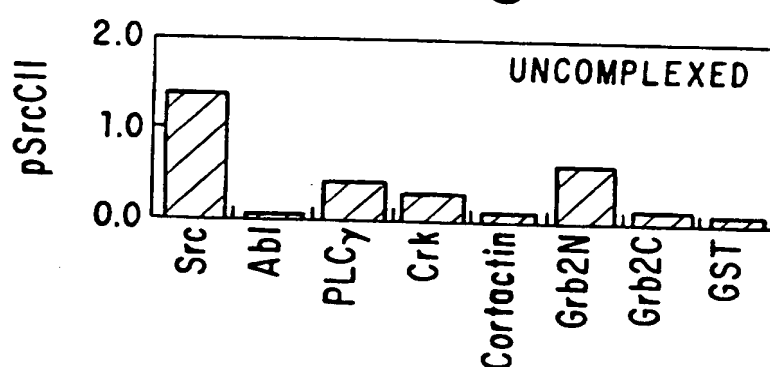


FIG. 8E

10/61

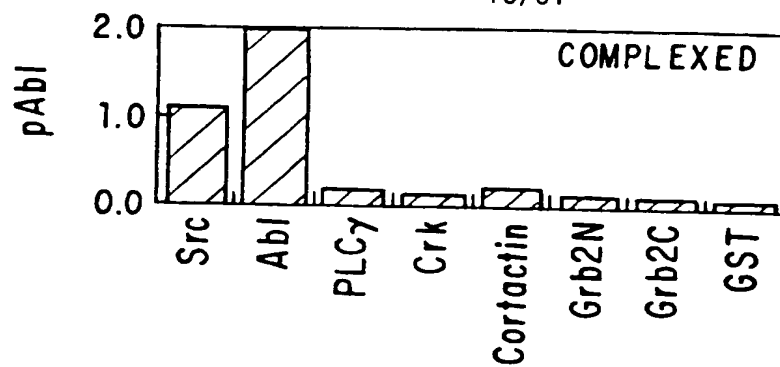


FIG. 8F

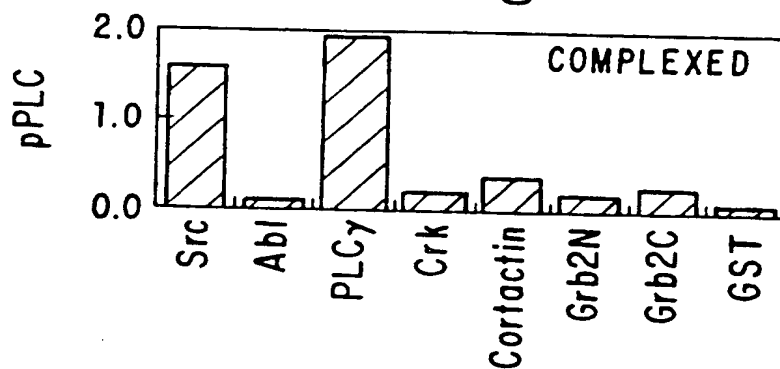


FIG. 8G

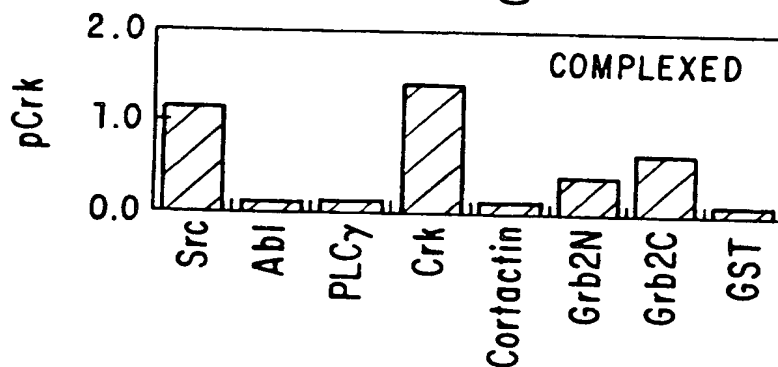


FIG. 8H

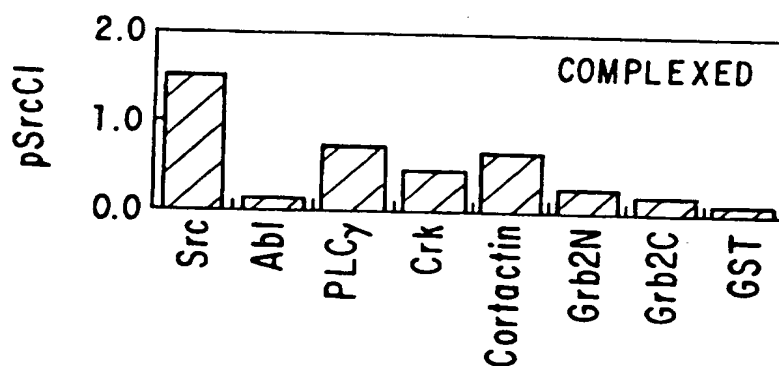


FIG. 8I

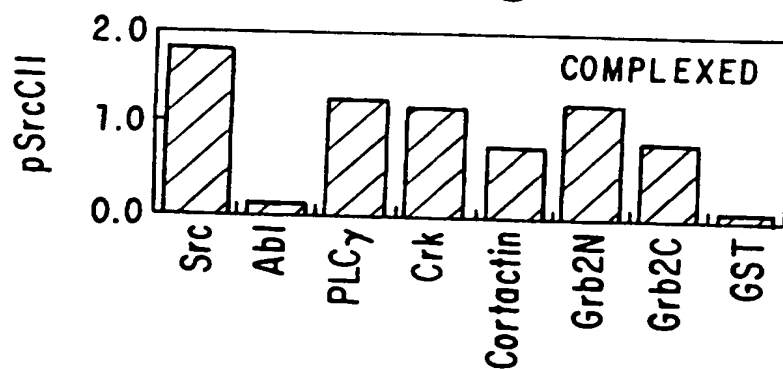


FIG. 8J

SUBSTITUTE SHEET (RULE 26)

11/61

LIGAND: pSrcCII pCort pSrcCII pSrcCII  
LIBRARY: Mouse Human Human 2

NAME	IDENTITY	#CLONES
ARAR	SH3P1 p53bp2	1
PHSH3ARARAR	SH3P2 Novel	1
SH3	SH3P3 Novel	1
HELIXP	SH3P4 Novel	1
CRICRRCRRCR	SH3P5 Cortactin	2
LIMNRNR	SH3P6 MLN50	3
Drebrin HELIX	SH3P7 Novel	5
HELIXP	SH3P8 Novel	5
Amphiphysin	SH3P9 Novel	9
CRICRRCR	SH3P10 HS1	1
SH2	SH3P11 Crk	2
Sorbin	SH3P12 Novel	1
HELIXP	SH3P13 Novel	2
SH3	SH3P14 H74	8
SH3 SH2	SH3P15 Lyn	1
SH3 SH2	SH3P16 Fyn	2
SH3 SH3 SH3	SH3P17 Novel	1
SH3 SH3 SH3	SH3P18 Novel	2

FIG. 9

SUBSTITUTE SHEET (RULE 26)



12/61

SH3P1	p53bp2	NKGTVALWDYEAQNSDELSFHGDAITILRRKDEN..	ETEMMARLG.....	DREGVVPKNLLGLY
SH3P2	Novel	QVKVFRALYTFEPTPDEL YFEEDIIYITDMS.....	DTSMWKGTK.....	GRTGLIPSNVVAEQ
SH3P3	Novel	HWTPYRAMYQYRPONEDELELREGDRVDMQOC.....	DDGWFGVSRRT.....	QKFGTFPGNYVAPV
SH3P4	Novel	DQPSCKALYDFEPENDGELGREGDLITLTNQI.....	DENWYEGMLH.....	GQSGFFPLSYVQVL
SH3P5	Cortactin	LGITAIALYDYQAAGDDEISFPDDIITNIEMI.....	DDGWRGVCK.....	GRYGLFPANYVELR
SH3P6	MLN50	GGKRYRAVDYDYSAADEDEVSFQDGTIVNVQI.....	DDGMYGTVERT.....	GDTGMLPANYVEAI
SH3P7	Novel	QGLCARALYDYQAADDTEISFDPENLITGIEVI.....	DEGWRGYGPD.....	GHFGMFPANYVELI
SH3P8	Novel	DQPCCRALYDLEPENEGELAFKEDIITLTNQI.....	DENWYEGMLH.....	GQSGFFPINYVEIL
SH3P9	Novel, m	FMFKVQAQHDYATATDDELQKAGDWLVIPFQNP	EEQDEGMLGVKESDWNQHKLEKCRGVFPENFTERV	
	Novel, h	FMFKVQAQHDYATATDDELQKAGDWLVIPFQNP	EEQDEGMLGVKESDWNQHKLEKCRGVFPENFTERV	
SH3P10	HS1	AGISAIALYDQGESELSFDPDDIITDIEMV.....	DEGWRGQCR.....	GHFGLFPANYVKLL
SH3P11	Crk A	EAEYVRALDFNGNDEEDLPFKKGDIIRDKP.....	EEQWNAEDSE.....	GKRGMPVPYVEKY
	B	RVIOKRVPNAYDKTALALEVGLVKVTKINV.....	SGQWEGECN.....	GKRGHFFFTVRL
SH3P12	Novel A	EMRPARAKDFKAQTLKELPQKGDVVYIRQI.....	DQNWYEGEHH.....	GRVGIFPRTYIELL
	B	EYGEIAKFNFGDTQVEMSFRRGERITLLRQV.....	DENWYEGRIPGT.....	SRQGIFFITYVDVL
	C	DLCSYOALYSYVPQNDDELELRGDIVDVMEKC.....	DDGMFVGTSRRT.....	RQFGTFPGNYVKPL
SH3P13	Novel	DQPCCRGLYDFEPENEGELGFKEGDIITLTNQI.....	DENWYEGMLR.....	GESGFFPINYVEVI
SH3P14	H74, m	TEVRVRALDYEGQEHDELSFKAGDELTKMEDE.....	EOGWCKGRLDN.....	GQVGLYPANYVEAI
	H74, h	KGVRVRALYDYGQEQDEL SFKAGDELTKLGEED.....	EQGWCRGRIDS.....	GQLGLYPANYVEAI
SH3P15	Lyn	QGDIVVALYPYDGIHPDDL SFKKGEKMKVLEE.....	HGEWKKAKSLLT.....	KKEGFIIPSNYVAKL
SH3P16	Fyn	GVTLFVALYDYEARTEDDLSFHKGEKFQILNSS.....	EGDWMEARSLTT.....	GETGYIPSNYVAPV
SH3P17	Novel A	KVYYRVALYPFESRSHDEITIQPGDIVMVDESQTG.....	EPGWLGGELK.....	GKTGMFPANYAEKI
	B	EGLQAQALYPWRACKDNHLNFKNDVITVLEQ.....	QDMWFGVQ.....	GQKGWFPKSYVKLI
	C	GEEIAQVIASTATGPEQLTLAPGQLILIRKKN.....	PGGWMEGELQARGKK.....	RQIGWFPANYVKLL
	D	AVCQVIAMVDYTAQNDDDELAFNKGQIINVLNKE.....	DPDWMKGEVN.....	GQVGLFPSNYVKLT

FIG. 10A

SUBSTITUTE SHEET (RULE 26)

13/61

SH3P18 Novel B VGEYIALYPYSSVEPGDLTFTEGEEILVTQK.....DGEWWTGSIG.....DRSGIFPSNYVKPK  
C KPEIAQVTSAYVAGSEQLSLAPGQLILILKKN.....TSGWQGEIQARGKK.....RQKGWFPASYVKLL  
D PVCQVIGMYDYAANNEDELSFSKGQLINVMNKD.....DPDWQGEIN.....GVTGLFPSNYVLEE

Src GVTTFVALYDYESRTETDLSFKKGERLQIVNNT.....EGDWMLAHSLLT.....GQTGYIPSNYVAPS

FIG. 10B

SUBSTITUTE SHEET (RULE 26)

14/61

CLONE 5 P3-6	QVKVFRALYTFEPRTPDELYEEGDIIYITDM	DTNWKGTS	GRTGLIPSNVVAEQ
CLONE 34 Crk LIKE	TGEEYIANGDFTAQVGDLTFFKKEILLVIEKK	PDGWIADAK	GNEGLVPRTYLEPY
CLONE 40 Ab1 BINDING PROTEIN	YLEKVVAIYDYTKDELSFQEGAIYVIKKN	DDGWYEGVMN	GVTGLSPGNVYESI
CLONE 41 Nck LIKE A	LNIPAFVKFAYVAIEREDELVLKGSRTVMKEC	SDGWRGYSN	GQIGWFFPSNYVLEE
Nck LIKE B	VLHVVTQLYPFSSVTEELNEFEKGETMEVIEKPENDPEWCKCKNAR		GQVGLVPKNYVVVL
CLONE 45 Nck A	EEVVVAKFDYVAQQEQELDIKKNERLWLLDD	SKSWVRVNSM	NKTGFVPSNYVERK
Nck B	LNMPAYVKFNMAEREDELVLKGTIVMEKIC	SDGWRGYSN	GQVWGFPSNYVTEE
Nck C	VLHVVOALYPFSSNDEELNFEKGDVMDVIEKPEN	DPEWVKCRKIN	GMVGLVPKNYVTVM
CLONE 53 NAB	DLFSYQALYSYIPQNDDELELRDGDIVDMKEC	DDGWFGVTSRRT	KQFGTFPGNYVKPL
CLONE 55 NOVEL	QGRKERARYDLEAAQDNELTFKAGEIMTVLDDS	DPNIWKGETH	QGIGLFPSNFTAD
CLONE 56 NOVEL	QGLCARALYDYQAADDTESISFDPENLITGIEVI	DEGWWRGYGPD	GHFGMFPANYVELI
CLONE 65 NOVEL A	VLVNRALYPFEARNHDEMFSNSGDI IQVDEKTVG	EPGWL YGSFQ	GNFGWFFPCNYVEKM
B	VENLKAQALCSWTAKK DNHLNFSKHDIITVLEQQ	ENFWWFGEVH	GGRGWFPKSYVKII
C	VGEYIALYPYSSVEPGDLTFTEGEEILVTQK	DGEWWTGSIG	DRSGIFPSNYVKPK
D	KPEIAQVTSAYVASGS EQLSLAPGQLILILKKN	TSGWVQGEIQARGKKRQKGFASWVKLL	
E	PVCQVIAMYDYAANNEDELVSFGQL INVMNKD	DPDWVQGEIN	GVTGLFPSNYVKMT

FIG. 10C

SUBSTITUTE SHEET (RULE 26)

15/61

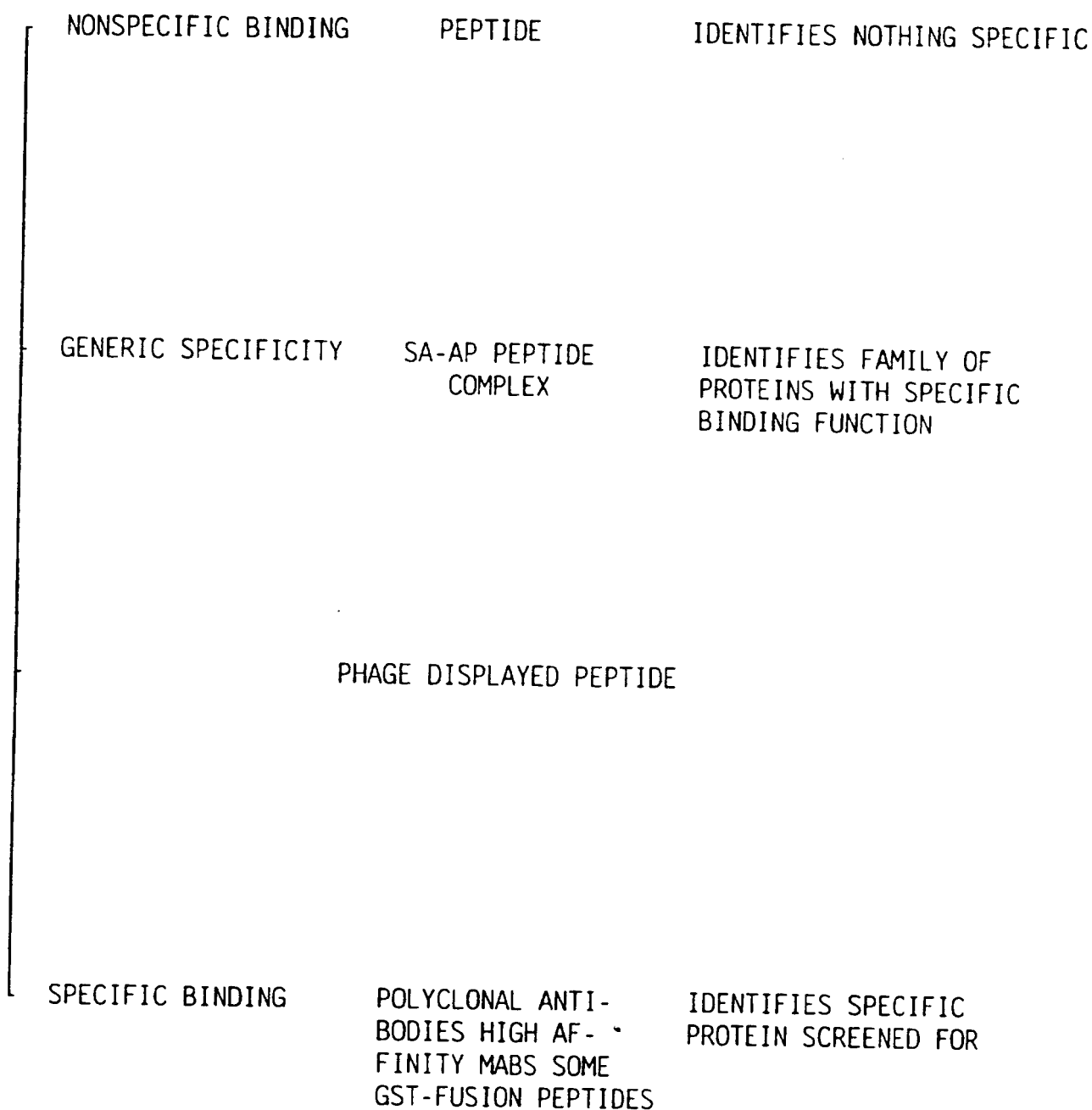


FIG. 11

SUBSTITUTE SHEET (RULE 26)

16/61

## SH3 DOMAIN CLONES

PEPTIDE	NAME	SEQUENCE	5	11	12	13	14	18	34	40	41	45	46	53	55	56	65
SH3001	WBP-1	PGTPPPPYTVGPGY	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TPPY	WBP-1	HGTPPPPYTVGP	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
QPPY	WBP-2	YVQPPPPYPGPM	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
YPPE	WBP-2	PGYPPPPEFY	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
WW005	WBP-1	PGTPAPPYTVGPGY	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
WW006	WBP-1	PGTPAPPYTVGPGY	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
bSH3002	K+ CHANNEL	DSGVRPLPLPDPCV	-	-	-	+	+	-	++	-	-	-	-	-	-	-	-
bSH3003	K+ CHANNEL	VRPLPLPEELPRRRPPPED	-	-	+	+	+	-	++	-	-	-	-	-	-	-	-
bSH3004	M4 AChr	PPPALPPPRPVADK	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
bSH3005	$\beta$ 1 ADRENERGIC	APAPPCPPPPAAAA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
bSH3006	RosGop	GGGFPLPPPYLPPLG	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
bSH3007	MEK	SISPRPRPPGPRVSG	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
bSH3008	P Tyr PHOSP.	PPPEHIPPMPKRIIE	-	-	+	-	-	++	-	+	-	-	-	-	-	-	-
bSH3009	Fak	KEGERALPSIPKLAN	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-
bSH3010	c-AbI	SRLKPAPPPPPAASAG	-	-	-	-	-	++	-	-	-	-	-	-	-	-	-
bSH3011	c-CbI	QASLPPVPPRDLLP	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
bSH3012	c-CbI	PVPPTLRDLPPPPPPDRPYS	-	-	+++	++	++	-	+	-	-	-	-	-	-	-	-
bSH3013	Co2+ CHANNEL	SDGGRNLPGIPVAS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
bSH3014	Co2+ CHANNEL	RHSRRQLPPVPPKPRLL	-	-	+	+	+	-	-	+	-	-	-	-	-	-	-
bSH3015	Nef	EKVGFPVTPQVPLRPMY	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
bSH3016	MUS CADHERIN	PQPHRVLPISPSDIA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
bSH3017	AP2	ADFQPPYFPPTYQPTYPQS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
bSH3018	ACTIN BINDING	SSAAPPPPPRRATPEK	-	+	+++	-	-	+++	-	+++	-	-	+	+++	-	-	-

FIG.12A

SUBSTITUTE SHEET (RULE 26)

bSH3019	EZRIN	SKKGWMTAPPPPPPVVPCG
bSH3020	VINCULIN	EAFQPQEPDFPPPPDLE
bSH3021	VINCULIN	DELAPPKPLPEGVPPPRPPPPE
bSH3022	DYNAMIN	PORRAPVPVPPARPCSR
bSH3023	DYNAMIN	LGCAPPVPSRPGASPDG

NUMBER	DOMAINS ENCODED BY CLONE															
1	4	3	1	1	1	1	1	2	3	1	1	1	1	1	1	5

**FIG. 12B**

**SUBSTITUTE SHEET (RULE 26)**

18/61

## SH3 DOMAIN CLONES

PEPTIDE	NAME	SEQUENCE	5	11	12	13	14	18	34	40	41	45	46	53	55	56	65
bSH3024	FasAqLi <sub>q</sub>	PPPLPPLPLPLKKRGNH	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
bSH3025	Rb	AAEPPAPPPPPPEDPGG	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
bSH3026	Rb	DEEVNIPPHIPVTV	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
bSH3027	Rb	SAEGSNPPKPLKKLRFD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
WW001	DYSTROPHIN	AWMGSPPEEGWF	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
WW002	DYSTROPHIN	AEWLEGPPWDRKEGF	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
WW003	DYSTROPHIN	GLEGWYMERGW	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
WW004	DYSTROPHIN	WGLDGLVDGWS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T12SRC1	Src	GILAPPVPRNTR	-	++	+	++	++	++	-	++	-	-	++	++	-	-	++
T12SRC4	Src	VLKRPLPIPPVTR	-	-	+	+++	+++	+++	+++	+++	-	-	-	-	-	-	++
T12SRC4M	Src	VLKRPLPPLVTR	-	-	+++	+++	+++	+++	+++	+++	-	-	-	-	-	-	++
NCKS1/4	Nck	SRSLSVSKPPIRSVLSR	-	-	-	-	-	-	-	+++	+++	+++	-	-	-	-	++
ABL G1/2M	c-Ab1	SRPPRWSPPVPLPTSLDSR	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CORT.M4	CORTACTIN	SRLGEFSKPPIPQKPTWMSR	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P53BP2.CO	P53 Bd PROT.	SFAAPARPVPVRKSRPCC	-	+++	+	+	+	+	+	+	+	+	+	+	+	+	++
P53BP2.P5	P53 Bd PROT.	SYDASSAPQRPLPVKSRPCC	-	+++	+	+	+	+	+	+	+	+	+	+	+	+	++
PLCG.CON	PLC- $\gamma$	SPPVPPRPATLGG	-	+++	+	+	+	+	+	+	+	+	+	+	+	+	++
CRK.CON	Crk	SVAPPPPLPKSGG	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NCK1.CON3	Nck	SFSFPLPPAPCC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NCK2.CON	Nck	SVPLPPLRTVSLGG	-	-	-	-	-	-	-	+++	+	++	-	-	-	-	-
NUMBER OF DOMAINS ENCODED BY CLONE			1	4	3	1	1	1	1	1	2	3	1	1	1	1	5

FIG.13

SUBSTITUTE SHEET (RULE 26)

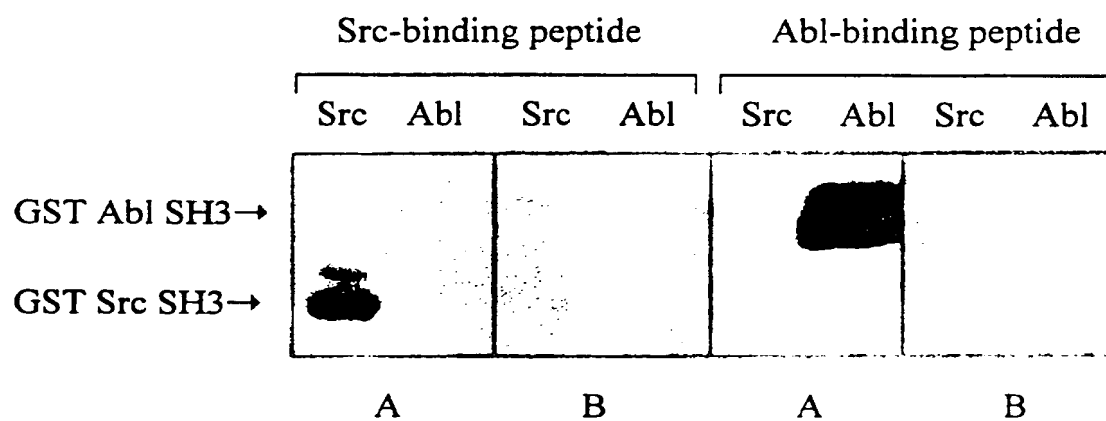


FIG.14

SUBSTITUTE SHEET (RULE 26)



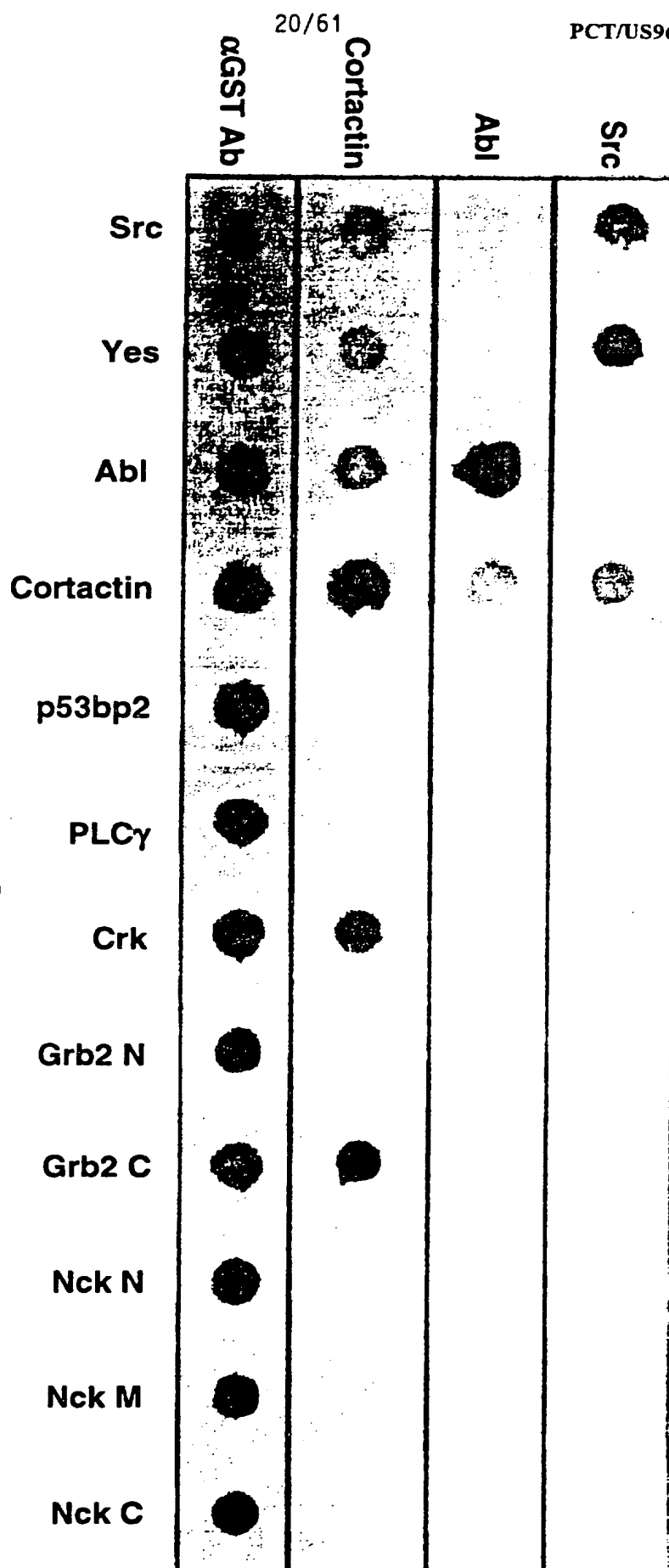
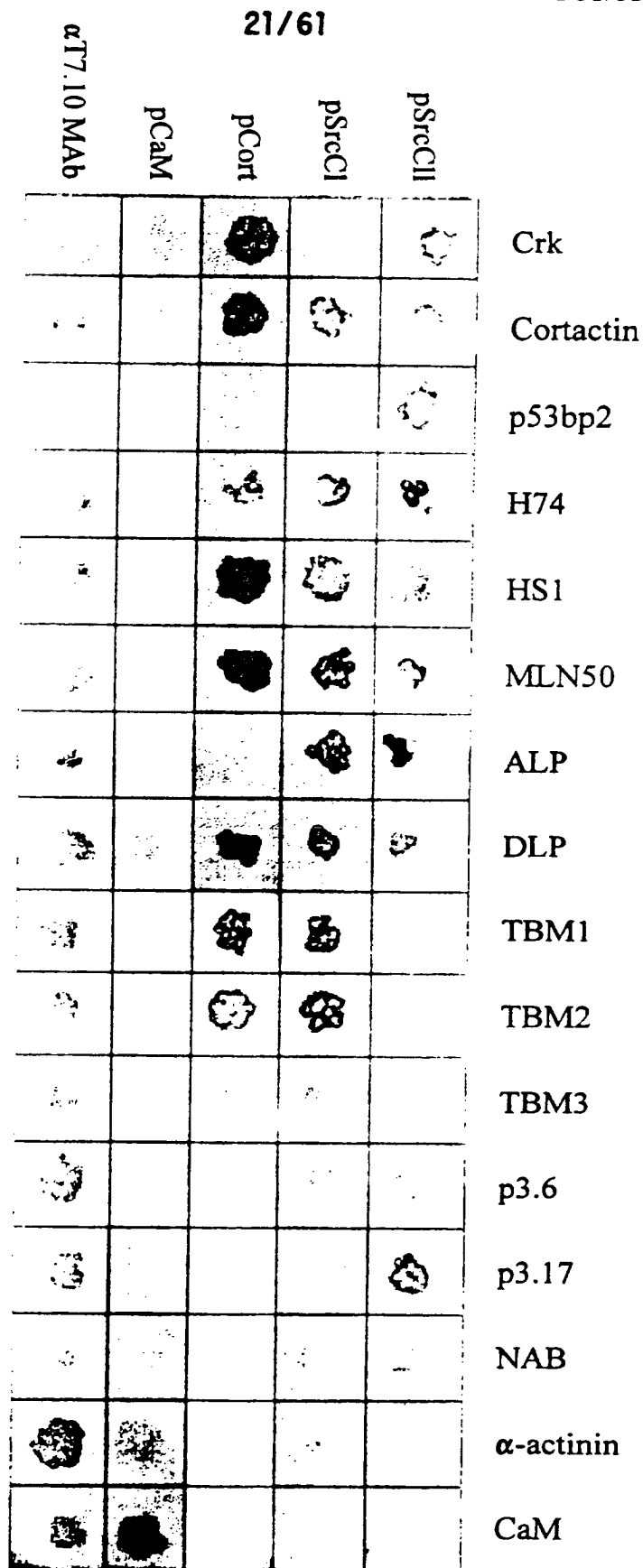


FIG.15

SUBSTITUTE SHEET (RULE 26)

FIG. 16



SUBSTITUTE SHEET (RULE 26)

22/61

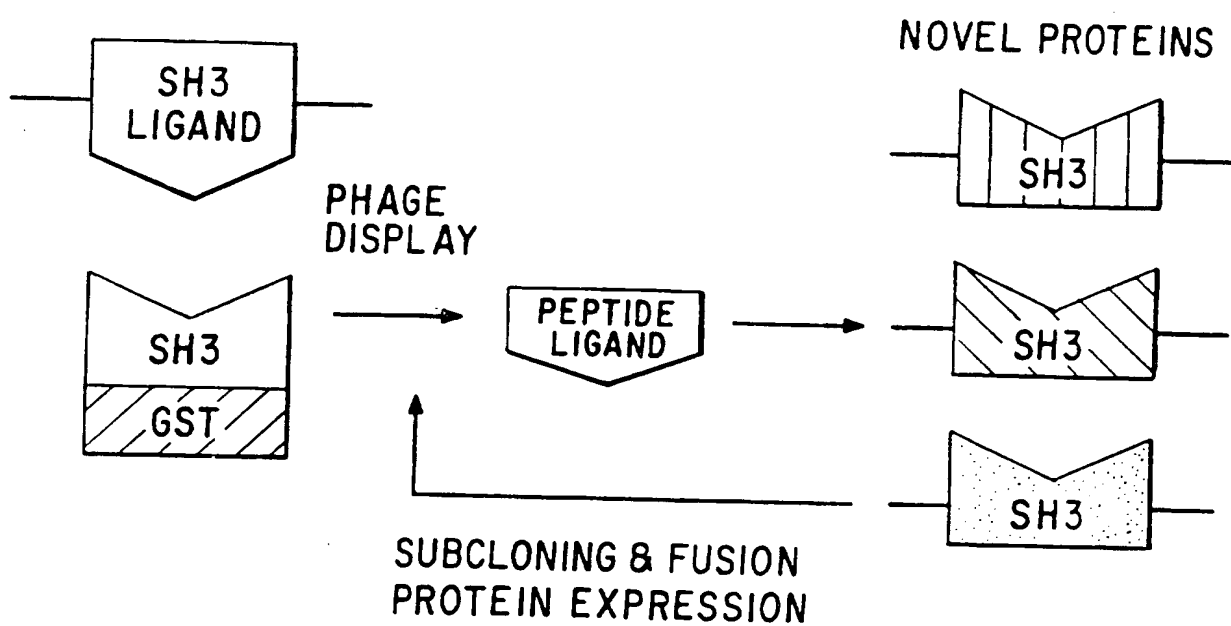


FIG. 17

SUBSTITUTE SHEET (RULE 26)

23/61

1 GTGAATGCTG CAGACAGTGA CGGATGGACA CCACTGCATT  
41 GTGCTGCCTC TTGCAACAGT GTCCACCTCT GCAAGCAGCT  
81 GGTGGAAAGT GGAGCCGCTA TCTTTGCCTC CACCATCAGT  
121 GACATTGAGA CTGCTGCAGA CAAGTGTGAA GAGATGGAAG  
161 AGGGATACAT CCAGTGTTCC CAGTTTCTGT ATGGGGTACA  
201 AGAGAAGCTG GGAGTGATGA ACAAAGGCAC CGTGTATGCT  
241 TTGTGGGACT ACGAGGCCCA GAACAGCGAT GAGCTGTCCT  
281 TCCATGAAGG GGATGCCATC ACCATCCTGA GGCGBAAAGA  
321 TGAAAACGAG ACCGAGTGGT GGTGGGCTCG TCTTGGGGAC  
361 CGGGAGGGCT ACGTGCCCAA AAATTGCTG GGGTTGTATC  
401 CACGGATCAA ACCCGGGCAG CGAACACTTG CCTGAACCCC  
441 CTGGAGTACC ACAGTCTCGT TTGCTCCAG GAGCTACTGG  
481 AGGAGATCCC ACTGCCCTGG GAAACTGAA GCTAGGATGG  
521 TCTCCTGGTG CTCACTTTAG CAGACAGTGT CCACAATGTG  
561 AATCCCACTT CCCAGGTGAG GCCCTCTCCA GGCTGCAGGA  
601 GCTGG

FIG. 18

1 VNAADSDGWT PLHCAASCNS VHLCKQLVES GAAIFASTIS  
41 DIETAADKCE EMEEGYIQCS QFLYGVOEKL GVMNKGTVYA  
81 LWDYEAQNSD ELSFHEGDAI TILRRKDENE TEWWARLGD  
121 REGYVPKNLL GLYPRIKPRQ RTLA

FIG. 19

1 SGCARSGAAA ASAGLAPSCR VRVGLPRLSL VAPCSAMSKP  
41 PPKPVKPGQV KVFALYTFE PRTPDELYFE EGDIIYITDM  
81 SDTSWWKGTC KGRTGLIPSN YVAEQAESID NPLHEAAKRG  
121 NLSWLRECLD NRVGVNGLDK AGSTALYWAC HGGHKDIVEV  
161 LFTQPNVELN QQNKLGDTAL HAAAWKGYAD IVQLLLAKGA  
201 RTDLRNNEKK LALDMATNAA CASLLKKKQQ GTDGARTLSN  
241 AEDYLDEDS D

FIG 21

SUBSTITUTE SHEET (RULE 26)

24/61

1     ..GAATTCAA GCTCGGGTTG CGCGCGGTCC GGAGCGGCCG  
41    CGGCCAGCGC AGGCTTGGCG CCCAGTTGTC GTGTGCGTGT  
81    GGGGCTCCCG CGGCTGAGCC TGGTCGCTCC GTGTAGCGCC  
121   ATGTCCAAGC CACCTCCCAA ACCGGTCAAA CCAGGGCAAG  
161   TTAAAGTCTT CAGAGCTCTA TATACATTTG AATCCAGAAC  
201   TCCAGATGAA TTATACTTTG AAGAAGGAGA CATTATCTAC  
241   ATCACTGACA TGAGTGATAC CAGCTGGTGG AAAGGGACAT  
281   GCAAGGGCAG AACAGGACTG ATCCCAGACA ACTATGTGGC  
321   TGAGCAGGCA GAATCCATTG ACAATCCATT GCATGAAGCT  
361   GCAAAAAGAG GCAACCTGAG CTGGTTGAGG GAGTGCTTGG  
401   ACAACCGGGT GGGTGTGAAC GGCCTGGACA AAGCTGGAAG  
441   CACAGCCCTG TACTGGGCCT GCCACGGTGG CCATAAAGAC  
481   ATAGTGGAGG TTCTGTTTAC TCAGCCGAAT GTGGAGCTGA  
521   ACCAGCAGAA TAAGCTGGGA GACACAGCTC TGCACGCGGN  
561   TGCCTGGAAG GGTTATGCAG ACATTGTCCA GTTGCTACTG  
601   GCAAAAGGTG CGAGGACAGA CTTGAGAAAC AATGAGAAGA  
641   AGCTGGCCTT GGACATGGCC ACCAACGCTG CCTGTGCATC  
681   GCTCCTGAAG AAGAAGCAGC AGGGAACAGA TGGGGCTCGA  
721   ACGTTAAGCA ACGCCGAGGA CTACCTCGAT GACGAAGACT  
761   CAGACTGATT CCCCCCGGGG CCGCTTTGAT TGTTGCCTAA  
801   ACTTCTTTTG CTTTTGCCAT TCCGGAGCCT GGGTTGTTTG  
841   CCAGAAGAGT ATTGATAACT GTTGCTTTTA AAGTCTGTAT  
881   GAGCGCGACA CTGCTGCACT GTGATCTGTG AGGAGTCGTT  
921   GTGAGGGTGG CTCATTCTCA CCCACGCCTT GNCAATAAGT  
961   GAAGAGATAC TTTGTTGTAT AAAATACATA TATGCTCACC  
1001  AGGGTAAAAT AAACGAAAAA AANTTATTTT TATTTATCAA  
1041  GCTAAAAAAA AAAAGCTTGG GCCCTNTTCT ATAGTGTCAC  
1081  CTAAATACTA GCTTGANCCG GNTGCTAACA AAGCCCGAAA  
1121  GGAAGCTGAG TTGCTGCTGC CACCGNTGAG CAATAACTAG  
1161  CATANCCCTT TGGGGCCTCT AAACGGGTCT TGAGGGGTTT  
1201  TTNGNTGAAA GGAGGANCTA TTTCCGGATA ACCTGGNGTA  
1241  ATAGGAAGA GGCCGNACC GATCGCCCTT CCCAACAGA

FIG. 20

SUBSTITUTE SHEET (RULE 26)

## 25/61

1 .ACTCACGNC GGTGGAGTGG TACCGGATCG AATTCAAGCC GCATCACTGG  
 51 CACTGGACGC CAGGGCATCT TCCCTGCCAG CTACGTGCAG ATAAACCGAG  
 101 AGCCCCGGCT CAGGCTTTGT GATGATGGTC CCCAGCTCCC TGCATCACCT  
 151 AACCCGACAA CCACTGCTCA CCTAAGCAGC CACTCCCACC CCTCCTCAAT  
 201 ACCTGTGGAC CCCACTGACT GGGGAGGTCG AACCTCCCCT CGACGCTCCG  
 251 CCTTTCCCTT CCCCATCACC CTCCAGGAGC CCAGATCCCA AACCAGAGT  
 301 CTCAATACCC CTGGACCAAC CCTGTCCCAT CCTCGAGCCA CCAGCCGTCC  
 351 CATAAACCTG GGACCCTCCT CCCCAAACAC AGAGATACAC TGGACTCCGT  
 401 ACCGGGCCAT GTACCAGTAC AGGCCCCAGA ATGAGGACGA GCTGGAAGTT  
 451 CGAGAGGGGG ACCGTGTGGA TGTCATGCAG CAATGTGACG ATGGCTGGTT  
 501 TGTGGGTGTC TCCCGGCGAA CTCAGAAATT TGGGACATTC CCTGGAAATT  
 551 ATGTAGCCCC AGTGTGAGTG GTCTCCATGG CAGTTTGGAG CCAACGAGGA  
 601 TCGGGAGGGG AGCAGTAGCA CTATGGGAGG GAGAGAGGCC TTCCATAGCC  
 651 TCCTCCCCAG GACCTGTGCT CCCAGCTTCT GCAGAGACCC CAGCAACTTT  
 701 CCCTCCAAGC CTCCTTGAAG TCGATTCCC ACCCCGCAAG TCACAGGCAT  
 751 TCCTTTGACA GCCCCCTTCA CCGCCCCTCA AATACAGACA TCTGCTTTCA  
 801 TGTGGGNAAA AAAAAAAT TAAAGGTGG CCCTAT

## FIG.22

1 RITGTGROGI FPASYVQINR EPRLRLCDDG PQLPASPNPT  
 41 TTAHLSSHSH PSSIPVDPTD WGGRTSPRRS AFPFPITLQE  
 81 PRSQTQSLNT PGPTLSHPRA TSRPINLGPS SPNTEIHWTP  
 121 YRAMYQYRPQ NEDELELREG DRVDVMQQCD DGWFGVSRR  
 161 TQKFGTFPGN YVAPV

## FIG.23

1 MSVAGLKKQF HKATQKVSEK VGGAEGTKLD DDFKEMERKV  
 41 DVTSRAVMEI MTKTIEYLQP NPASRAKLSM INTMSKIRGQ  
 81 EKGPGYPQAE ALLAEAMLKF GRELGDDCNF GPALGEVGEA  
 121 MRELSEVKDS LDMEVKQNF1 DPLQNLHDKD LREIQHHLKK  
 161 LEGRRLDFGY KKKRQGKIPD EELRQALEKF DESKEIAESS  
 201 MFNLLEMDIE QVSQLSALVQ AQLEYHKQAV QILQQVTVRL  
 241 EERIRQASSQ PRREYQPKPR MSLEFATGDS TOPNGGLSHT  
 281 GTPKPPGVQM DQPCCRALYD LEPENEGELA FKEGDIITLT  
 321 NQIDENWYEG MLHGQSGFFP INYVEILVAL PH

## FIG.25

SUBSTITUTE SHEET (RULE 26)

26/61

1 TNNNNYYMM SKYSKKGKKK KGKWMGRCT GATTCAAGCC GACCAGCGGC  
 51 GGCCCGGCGA CCCAGCCGC CTCTCCGCAT CTGCATCTGC ATCTGCCGGC  
 101 CGCGCAGCCT CCCGCATCCC ATCATGTCCG TGGCAGGGCT GAAGAAGCAG  
 151 TTCCACAAAG CCACTCAGAA AGTGAGTGAG AAGGTGGGAG GAGCGGAAGG  
 201 CACCAAGCTC GATGATGACT TCAAAGAGAT GGAGAGGAAA GTGGATGTCA  
 251 CCAGCAGGGC TGTGATGGAG ATAATGACAA AAACGATTGA ATACCTCCAA  
 301 CCCAATCCAG CTTCCAGGGC TAAGCTCAGT ATGATCAACA CCATGTCGAA  
 351 AATCCGCGGC CAAGAGAAGG GGCCAGGCTA CCCTCAGGCG GAAGCACTGC  
 401 TGGCAGAGGC CATGCTCAAG TTCGGCAGGG AGCTGGGTGA TGATTGCAAC  
 451 TTTGGTCCTG CTCTCGGTGA GGTGGGAGAA GCCATGAGGG AGCTCTCGGA  
 501 GGTCAAGGAC TCATTGGACA TGAAGTGAA GCAGAAATTC ATCGACCCCC  
 551 TTCAGAATCT TCATGACAAG GATCTGAGGG AGATTCAGCA TCATCTGAAA  
 601 AAGCTGGAAG GCCGACGCTT AGACTTTGGT TATAAGAAGA AGCGACAAGG  
 651 CAAGATTCCA GATGAAGAAC TCCGCCAAGC TCTGGAGAAA TTCGATGAGT  
 701 CTAAAGAAAT CGCCGAGTCG AGCATGTTCA ACCTCTTGGA GATGGATATA  
 751 GAACAGGTGA GCCAGCTCTC CGCACTTGTT CAGGCTCAGC TGGAGTACCA  
 801 CAAGCAGGCA GTGCAGATCC TGCAGCAGGT CACTGTCAGA CTGGAAGAAA  
 851 GAATAAGACA AGCTTCATCT CAGCCAAGAA GGAATATCA GCCCAAACCA  
 901 CGGATGAGCC TAGAGTTTGC CACTGGAGAC AGTACTCAGC CCAACGGGGG  
 951 TCTCTCCAC ACAGGCACAC CCAAACCTCC AGGTGTCCAA ATGGATCAGC  
 1001 CCTGCTGCCG AGCTCTGTAT GACTTGGAAC CTGAAAATGA AGGGGAATTG  
 1051 GCTTTTAAAG AGGGCGATAT CATCACACTC ACTAATCAGA TTGACGAGAA  
 1101 CTGGTATGAG GGGATGCTTC ATGGCCAGTC TGGCTTTTTC CCCATCAACT  
 1151 ATGTAGAAAT TCTGGTTGCT CTGCCCCATT AGGATCCTGT GCTGGCTGGC  
 1201 TCACCTCCTT CTGACCCAGA TAGTTAAGTT TAACCACTGC TTTGGTAATG  
 1251 CTGCTTCCAA TACATCACGA ATGCAGGCCG CAGTGGATGA GTCACCAAGC  
 1301 CCACACGTGC CCTGGGTTGA CCCGTGTGCT CCTCCAGGAG ACGCGGTGAT  
 1351 AGATGGTATC TTCCAAGGCC AGTGGGCCTG GTACATGCTT TAAAACACCA  
 1401 TCTGAGACTA GCCAGGAGTC CCAGAACTGG CTTACAGTT CTCAGGAGGC  
 1451 TGTGGTTTCT GGTAACATGC CTGTGAACCA CATGGCAGAA AACTCTCTCT  
 1501 CACTGAAGAT ATTGTCTCTC ACCCAGGGGC CATCTCAAGG TCTCCAGTTC  
 1551 TCCATTTACA GAGGAGAAAG TCCTTTTGT TGCATTTCC CTTCTAAAT  
 1601 ATGTGAGTCA CAGAATTGTT GGCAAAAACA TCCCCTCACC AGCAAGATGT  
 1651 CTGCTGGTTT AAGCAACTTG GTCTCTTGAT GCCATTAGCA AAAGTATTAA  
 1701 TTGTCCAAAG CACCTTTGTT CACTAATATC TATCTATCTA TCTATCTATC  
 1751 TATCTATCTA TCTATCTATC TATCTATCAT CTATCTACCT ACCTATCTAC  
 1801 CTATCATCTA TCTATCTATC ATCTATTATC TATCTATCTA TCTATCTATC  
 1851 NNTCNATCTA TCTATCTATC CATCTATCTA TCCATCATCT ATCTACCTAC  
 1901 CTATCTACTA TCCATCTATC TATCTATCCA TCATCTATCT ACCTACCTAT  
 1951 CTACTATCCA TCCATTTATC TATCTATCTA TCTATCTATC TATCTATCTA  
 2001 TCTCCCTCAT ACTTCTGAGA CATGGCCAGT TTTCTTCCCT CCCTGCTGTT  
 2051 AAGCACTTGG NAGATGAGGG GGGGGTCCC ATTTNATTTT TGAGTGAGAT  
 2101 GGTGAGCAGG GTGTATGTTG GCTGTNNTNN GGGGGTGGCC CTA

**FIG. 24**  
**SUBSTITUTE SHEET (RULE 26)**

27/61

1 CGGGCGCGGC GGGAGCCTGG TGGACCCTGC TTTGGCGGTA  
41 ATCATTGATC ATCGCAGATG CCCTCATATC CACTTTGGAT  
81 TCCTTGGATT CGGACAGACT CTGAACTGCT TTTCCCAGCA  
121 AAAGAGAAAAG ATGTGGAAAG CCTCTGCAGG CCATGCTGTG  
161 TCCATCACGC AGGATGATGG AGGAGCTGAT GACTGGGAGA  
201 CTGATCCTGA TTTTGTGAAT GATGTGAGTG AAAAGGAGCA  
241 GAGATGGGGT GCTAAAACCG TGCAGGGATC GGGGCACCAG  
281 GAACACATCA ACATTCACAA GCTTCGAGAG AATGTCTTCC  
321 AAGAACACCA GACGCTCAAG GAGAAGGAGC TGGAAACGGG  
361 ACCCAAGGCT TCCCACGGCT ATGGCGGGAA GTTCGGTGTG  
401 GAGCAGGATA GGATGGACAG ATCAGCCGTG GGCCATGAGT  
441 ACCAGTCGAA GCTTTCCAAG CACTGCTCAC AAGTGGACTC  
481 GGTCCGGGGC TTCGGAGGCA AGTTCGGTGT CCAGATGGAC  
521 AGGGTGGATC AGTCTGCTGT AGGCTTTGAA TACCAGGGGA  
561 AGACTGAGAA GCATGCCTCC CAGAAAGACT ACTCTAGTGG  
601 CTTCCGTGGC AAATACGGTG TGCAAGCTGA CCGTGTAGAC  
641 AAGAGTGCCG TGGGCTTTGA CTACCAGGGC AAGACGGAGA  
681 AGCATGAGTC TCAGAAAGAT TACTCCAAAG GTTTTGGTGG  
721 CAAATATGGG ATTGACAAGG ACAAGGTGGA TAAAGTGCT  
761 GTGGGCTTTG AGTATCAAGG CAAGACAGAG AAGCACGAAT  
801 CCCAGAAAGA CTATGTAAAA GGCTTTGGAG GAAAGTTTGG  
841 TGTGCAGACA GACAGACAGG ACAAGTGTGC CTTGGCTGG  
881 GACCATCAGG AGAAGCTGCA GCTGCATGAA TCCCAAAAAG  
921 ACTATAAGAC TGGTTTCGGA GGCAAATTTG GTGTTCACTC  
961 CGAGAGGCAG GACTCCTCCG CTGTGGGGTT TGATTACAAG  
1001 GAGAGATTGG CCAAGCACGA GCCCCAGCAA GACTATGCCA  
1041 AAGGATTCGG CGGGAAGTAT GGGGTGCAGA AGGATCGGAT  
1081 GGACAAGAAT GCATCCACCT TTGAAGAAGT GGTCCAGGTG  
1121 CCATCTGCCT ATCAGAAGAC TGCCCCATT GAGGCCGTAA  
1161 CCAGCAAAAC CAGTAATATC CGTGCTAACT TTGAAAACCT  
1201 GGCAAAGGAG AGAGAGCAGG AGGACAGGCG GAAGGCAGAA  
1241 GCCGAGAGAG CTCAGCGGAT GGCCAAAGAA AGACAGGAGC  
1281 AGCAGGAGGC GCGCAGGAAG CTGGAAGAGC AAGCCAGAGC  
1321 CAAGAAGCAG ACGCCCCCTG CATCCCCTAG TCCTCAACCA  
1361 ATTGAAGACA GACCACCCTC CAGCCCCATC TATGAGGATG  
1401 CAGCTCCGTT CAAGGCCGAG CCGAGCTACC GAGGTAGCGA  
1441 ACCTGAGCCT GAGTACAGCA TCGAGGCCGC AGGCATTCTT  
1481 GAGGCTGGCA GCCAGCAAGG CCTGACCTAT ACATCAGAGC  
1521 CCGTGTACGA GACTACAGAG GCTCCTGGCC ACTATCAAGC  
1561 AGAGGATGAC ACCTACGATG GGTATGAGAG TGACCTGGGC  
1601 ATCACAGCCA TCGCCCTGTA TGACTIONAG GCTGCTGGCG

FIG. 26A

SUBSTITUTE SHEET (RULE 26)



28/61

1641 ATGATGAGAT CTCCTTTGAC CCTGATGACA TCATCACCAA  
1681 CATAGAAATG ATTGACGATG GCTGGTGGCG TGGGGTGTGC  
1721 AAGGGCAGAT ACGGGCTCTT CCCAGCCAAC TATGTGGAGC  
1761 TGCGGCAGTA GGGCTGCCAC CCAGAGCCTA CCGGCACCAG  
1801 CACAGGGTTC ACACTACAGA GCATCTGCGT GTGTTTGAGT  
1841 TGGTTTCTGC TTCCGTTTCT GTTTTTG

FIG. 26B

SUBSTITUTE SHEET (RULE 26)

29/61

1 MWKASAGHAV SITQDDGGAD DWETDPDFVN DVSEKEQRWG  
 41 AKTVQSGGHQ EHINIHLRE NVFQEHQTLK EKELETGPKA  
 81 SHGYGGKFGV EQDRMDRSV GHEYQSKLSK HCSQVDSVRG  
 121 FGGKFGVQMD RVDQSAVGFE YQKTEKHAS QKDYSSGFGG  
 161 KYGVQADRVD KSAVGFDYQG KTEKHESQKD YSKGFGGKYG  
 201 IDKDKVDKSA VGFEYQGKTE KHESQKDYVK GFGGKFGVQT  
 241 DRQDKCALGW DHQEKQLLHE SQKDYKTGFG GKFGVQSERQ  
 281 DSSAVGFDYK ERLAKHEPQQ DYAKGFGGKY GVQKDRMDKN  
 321 ASTFEEVVQV PSAYQKTVPI EAVTSKTSNI RANFENLAKE  
 361 REQEDRRKAE AERAORMAKE RQEQEEARRK LEEQARAKQ  
 401 TPPASPPSPQ IEDRPPSSPI YEDAAPFKA PSYRGSEPEP  
 441 EYSIEAAGIP EAGSQGLTY TSEPVYETTE APGHYQAEDD  
 481 TYDGYESDLG ITAIALYDYQ AAGDDEISFD PDDIITNIEM  
 521 IDDGWWRGVC KGRYGLFPAN YVELRQ

FIG. 27

1 AAGCAGTCCT TCACCATGGT GGCCGACACT CCGGAAAACC TCCGCCTCAA  
 51 GCAACAGAGC GAGCTGCAGA GTCAGGTGCG CTACAAGGAG GAGTTTGAGA  
 101 AGAATAAGGG CAAAGGTTTC AGCGTGGTGG CAGACACGCC TGAGCTGCAG  
 151 AGAATCAAGA AGACCCAGGA CCAGATCAGC AATATCAAAT ACCATGAGGA  
 201 GTTTGAGAAG AGCCGCATGG GGCCCACTGG AGGAGAAGGG GTGGAACCAG  
 251 AGCGCCGAGA AGCCAGGAC AGCAGCAGCT ACCGGAGGCC CACAGAGCAG  
 301 CAGCAGCCGC AGCCTCACCA TATCCCGACC AGTGCCCCCG TGTACCAGCA  
 351 GCCCCAGCAG CAGCAGATGA CCTCGTCCTA TGGTGGGTAC AAGGAGCCAG  
 401 CAGCCCCTGT CTCCATACAG CGCAGTGCCC CAGGTGGCGG TGGGAAACGG  
 451 TACCGTGCAG TGTATGACTA CAGCGCTGCC GACGAGGACG AGGTCTCCTT  
 501 CCAGGATGGG GACACCATCG TCAATGTGCA GCAGATCGAT GACGGCTGGA  
 551 TGTACGGGAC CGTAGAGCGC ACCGGTGACA CGGGGATGCT GCCAGCCAAC  
 601 TACGTGGAGG CCATCTGAAC CCTGTGCCGC CCCGCCCTGT CTTCAATGCA  
 651 TTCCATGGCA TCACATCTGT CCTGGGGCCT GACCCGTCCA CCCTTCAGTG  
 701 TCTCTGTCTT TTAAGATCTT CAACTGCTTC TTTATCCCCG CCCCTCCAGC  
 751 TTATTTTACC ATCCCAAGCC TTGTTCTGCC CCTGTCATGG GCTCCTTCCT  
 801 CTGGCAGGTT TTCCCTTGA CCAATCAACT GATTGATTTT TCTCTCTGGA  
 851 TGGAACAGGC TGGGCACTCT GGGGAGGGCA GGATTGTTCT TAGCTAGGTA  
 901 GACTCCCAGG GCTGGGCTGA ACTAGGAGAC CCACTAAGGA GATCAGTTTA  
 951 GACTGGGTGC AGTGGCAAAC ACCCTTAATT CCCAGCGAAG GGAGTCAGAG  
 1001 GCAGGCAGAT CTGTGACTTG GAAGCCAGCC TGGTCTACAT CGAGAGTTTC  
 1051 AGGACAGCCA GAGCTATGTA GTGAGGCCCT GTCTCGGAGG AAGAGTGGGG  
 1101 GTTGGTTAGC TCTCAGCTTC ACTTCCTGCC TTAGGCTCCT CAGAACCCCT  
 1151 GGCCAGCTC CCCCAACTCC CTTCTCCTA GAGGTGGGGT GAGCTGTGC

FIG. 28

SUBSTITUTE SHEET (RULE 26)

30/61

1 KQSFTMVADT PENLRLKQQS ELQSQVRYKE EFEKNKGKGF SVVADTPELQ  
51 RIKKTQDQIS NIKYHEEF EK SRMGPSGGEG VEPERREAQD SSSYRRPTEQ  
101 QQPQPHIPT SAPVYQQPQQ QOMTSSYGGY KEPAAPVSIQ RSAPGGGGKR  
151 YRAVDYSAA DEDEVSFQDG DTIVNVQQID DGWMYGTVER TGD TGMLPAN  
201 YVEAI

## FIG. 29

1 ATGGCGGTGA ACCTGAGCCG GAACGGGCGG GCGCTGCAGG AGGCCTACGT  
51 GCGCGTAGTC ACCGAGAAAT CCCCAGCCGA CTGGGCTCTT TTTACCTATG  
101 AAGGCAACAG CAATGACATC CGTGTGGCTG GCACAGGAGA GGGAGGCCTG  
151 GAGGAGCTGG TGAAGAGCT CAACAGCGGG AAGGTGATGT ACGCCTTCTG  
201 CAGGGTGAAG GACCCCAACT CCGGCCTGCC CAAGTTTGTC CTCATCAACT  
251 GGACAGGAGA GGGTGTGAAT GATGTGCGGA AAGGAGCATG TGCCAACCAC  
301 GTCAGCACCA TGGCCAACCT CCTGAAGGGT GCCCACGTGA CCATCAATGC  
351 CCGGGCCGAG GAGGATGTGG AGCCTGAGTG CATCATGGAG AAGGTTGCCA  
401 AGGCCTCTGG GGCCAACCTAC AGCTTCCATA AGGAAAGCAC CTCCTTCCAG  
451 GATGTAGGGC CGCAGGCCCC AGTGGGCTCT GTGTACCAGA AGACCAATGC  
501 CATATCTGAG ATCAAGAGAG TCGGCAAGGA TAACTTCTGG GCCAAAGCTG  
551 AGAAGGAAGA AGAGAACCGC CGCCTGGAGG AGAAGCGGCG TGCCGAAGAG  
601 GAGCGGCAGC GGTTGGAGGA GGAGCGACGA GAGCGGGAGC TGCAGGAGGC  
651 TGCCCCGACGT GAGCAGCGCT ACCAGGAACA GCACAGATCA GCTGGAGCCC  
701 CGAGCAGGAC AGGTGAGCCA GAGCAGGAAG CCGTTTCAAG GACCAGACAG  
751 GAGTGGGAGT CTGCTGGGCA GCAGGCCCCA CACCCACGAG AGATTTTCAA  
801 GCAGAAGGAA AGGGCAATGT CCACCACCTC TGTCACCAGC TCGAGCCGG  
851 GCAAGCTGAG GAGCCCCTTC CTGCAGAAGC AACTCACTCA ACCAGAAACC  
901 TCCTACGGCC GAGAGCCAC AGCTCCTGTC TCCCGGCCTG CAGCAGGTGT  
951 CTGTGAGGAG CCAGCGCCTA GCACTCTGTC TTCTGCCAG ACAGAAGAAG  
1001 AACCTACATA TGAAGTACCC CCAGAGCAGG ACACCCTCTA TGAGGAACCA  
1051 CCACTGGTAC AGCAGCAAGG GGCTGGCTCC GAACACATTG ACAACTACAT  
1101 GCAGAGCCAG GGCTTCAGTG GACAAGGGCT GTGCGCCCGG GCCTTGTATG  
1151 ACTACCAGGC AGCTGATGAC ACCGAGATCT CCTTTGACCC TGAGAACCTA  
1201 ATCACAGGCA TCGAGGTGAT TGACGAAGGC TGGTGGCGAG GCTATGGGCC  
1251 TGACGGCCAC TTTGGCATGT TTCCTGCCAA CTACGTGGAG CTCATAGAGT  
1301 GA

FIG. 30  
SUBSTITUTE SHEET (RULE 26)

31/61

1 MAVNLSRNGP ALQEAYVRVV TEKSPTDWAL FTYEGNSNDI RVAGTGEGGL  
51 EELVEELNSG KVMYAFCRVK DPNSGLPKFV LINWTGEGVN DVRKGACANH  
101 VSTMANFLKG AHVTINARAE EDVEPECIME KVAKASGANY SFHKESTSFO  
151 DVGPQAPVGS VYQKTNAISE IKRVGKDNFW AKAKEEENR RLEEKRAEE  
201 ERQRLEEERR ERELQEAARR EQRYQEQHRS AGAPSRTGEP EQEAVSRTRO  
251 EWESAGQQAP HPREIFKQKE RAMSTTSVTS SQPGKLRSPF LQKQLTQPET  
301 SYGREPTAPV SRPAAGVCEE PAPSTLSSAQ TEEPTYEVP PEQDTLYEEP  
351 PLVQQQGAGS EHIDNYMQSQ GFSGQGLCAR ALYDYQAADD TEISFDPENL  
401 ITGIEVIDEG WWRGYGPDGH FGMFPANYVE LIE

## FIG. 31

1 MSVAGLKKQF YKASQLVSEK VGGAEGTKLD DDFKDMKKV DVTSKAVAEV  
51 LVRTIEYLQP NPASRAKLTM LNTVSKIRGQ VKNPGYPQSE GLLGECMVRH  
101 GKELGGESNF GDALLDAGES MKRLAEVKDS LDIEVKQNF I DPLQNLCDK  
151 LKIEQHHLKK LEGRRLDFDY KKKRQKGIPD EELRQALEKF EESKEVAETS  
201 MHNLLDIE QVSQLSALVD AQLDYHRQAV QILEELADKL KRRVREASSR  
251 PKREFKPRPR EPFELGELEQ PNGGFPCAPA PKITASSSFR SSDKPIRMPS  
301 KSMPLDQPS CKALYDFEPE NDGELGFREG DLITLTNQID ENWYEGMLHG  
351 QSGFFPLSYV QVLVPLPQ

## FIG.33

1 MAEMGSKGVT AGKIASNVQK KLTRAQEKVL QKLGKADETK DEQFEQCVQN  
51 FNKQLTEGTR LQKDLRTYLA SVKAMHEASK KLSECLQEVY EPEWPGRDEA  
101 NKIAENNDLL WMDYHQKLVD QALLTMDTYL GQFPDIKSRI AKRGRKLVDY  
151 DSARHHYESL QTAKKKDEAK IAKAEELIK AQKVFEEMNV DLQEELPSLW  
201 NSRVGFYVNT FQSIAGLEEN FHKEMSKLNQ NLNDVLVSLE KQHGSNTFTV  
251 KAQPSDNAPE KGNKSPSPPP DGSPAATPEI RVNHEPEPAS GASPGATIPK  
301 SPSQPAEASE VVGGAQEPGE TAASEATSSS LPAVVVETFS ATVNGAVEGS  
351 AGTGRDLPP GFMFKVQAQH DYTATDTDEL QLKAGDVVLV IPFQNPEEQD  
401 EGWLMGVKES DWNQHKLEK CRGVFPENFT ERVQ

## FIG. 35

SUBSTITUTE SHEET (RULE 26)

## 32/61

1 TTNNCACTCA CCGTCCGTGG TNNNNSTMMC SGWYNKRNTK YRRKMSSKRW  
51 YKWKKCRRKS GCGGCGCCGA CCTGCGCGCG GAGGAAAGAA GTCGGTTCGG  
101 CGGCGCCGGC GGAAACCGGA GTTCGAGCGG GAGGCCTGAC GCGGCGAGGC  
151 GGCATGTCGG TGGCGGGGCT GAAGAAGCAG TTCTACAAGG CGAGCCAGCT  
201 GGTGAGCGAG AAGGTTGGTG GGGCCGAAGG GACCAAAGTG GATGATGACT  
251 TTAAAGATAT GGAAAAGAAG GTGGATGTCA CCAGCAAGGC CGTGGCAGAG  
301 GTGCTGGTCA GAACCATAGA ATATCTGCAG CCTAACCCAG CCTCGAGAGC  
351 CAAGCTGACT ATGCTGAACA CCGTATCCAA GATCCGGGGC CAAGTGAAGA  
401 ACCCTGGCTA CCCACAGTCA GAGGGTCTGT TGGGAGAGTG CATGGTTCGC  
451 CATGGCAAGG AACTAGGTGG AGAGTCCAAC TTCGGTGATG CCCTGCTAGA  
501 TGCAGGTGAG TCCATGAAGC GCCTGGCTGA GGTGAAGGAC TCACTGGACA  
551 TCGAGGTCAA GCAGAACTTC ATTGACCCAC TACAGAACCT GTGTGACAAG  
601 GATCTGAAGG AGATCCAGCA CCACCTGAAG AAATTGGAGG GCCGCCGCCT  
651 TGACTTTGAC TACAAGAAGA AGCGCCAGGG CAAGATCCCC GATGAGGAGC  
701 TGCGCCAGGC CCTAGAGAAG TTCGAGGAGT CCAAGGAGGT GCGGAGAGCC  
751 AGTATGCACA ACCTCCTGGA GACTGATATA GAGCAGGTGA GCCAGCTCTC  
801 GGCCCTGGTG GATGCCCAGC TGGACTACCA CCGGCAGGCA GTGCAGATCC  
851 TGGAGGAGCT GGCTGACAAG CTGAAGCGCA GGGTTCGGGA AGCCTCCTCA  
901 CGCCCCAAGC GGGAGTTCAA GCCCCGGCCC CGGGAGCCCT TTGAGCTTGG  
951 AGAGCTGGAG CAGCCCAATG GGGGATTCCC CTGTGCCCCA GCACCTAAGA  
1001 TCACAGCCTC CTCATCATTT AGATCGTCAG ACAAGCCCAT CAGGATGCCC  
1051 AGCAAGAGCA TGCCACCCCT GGACCAGCCA AGCTGCAAGG CGCTTTATGA  
1101 TTTTGAGCCA GAGAATGATG GCGAGCTGGG CTTCCGTGAG GGGGACCTCA  
1151 TCACGCTTAC CAACCAGATC GACGAGAACT GGTATGAGGG GATGCTGCAC  
1201 GGCCAATCAG GCTTCTTCCC ACTCAGCTAC GTGCAGGTGC TGGTGCCTCT  
1251 GCCTCAGTGA CTGGGCCTTT ACACCGCTGC CAGTCACAGT GCAGCAGCAG  
1301 TCTAATGCCA AGGTGCTCTA GAAACACTAA TGTTCTCCA GGGGGGACTC  
1351 CTCCCCACTC CCTCAGCCCT GGGGCCCCCC TATCCTAAGA CTCGGAAAGG  
1401 CCCACCCTGA GGTTCATTG CTTCTCTGGT GGTATCAGCT TCCAGCTGTT  
1451 TCAACCCTTC CCAGCCCGTT GCTGGCGATG GSCCNNGCC CCCTCTCTAG  
1501 GCTCTCTAGA GGCAGGCAGG TCCTTGGAAT CCCAGCCTG CAAGCAGAGG  
1551 CTGGCCAGCT CCCAGCTCA GCACACAGAC ACACCTGGCA CCTGCTGCTC  
1601 ATGAAGAAGT GCACAAGGCA CAAATGTGTA CACTTCCCAT GGGACCACAG  
1651 ACCCAGCTCA GCTCTGTTGA AGACCAAGCA CAAAGGCCTT GAAGAGTGGA  
1701 CATTCCCAGG TCCCTGGCAC CTTCCCTTGA GCCAGCTCCA TTGCTACTTA  
1751 TTCATGTGAC TGAAGCTGAC CACAGGCAGC TGGCAGGTCC TTTTTTCAAC  
1801 CAGCAGGCTA GGCTGGCCAT AGACCCAGCT CTGCCTCACC CTGCCATGTT  
1851 CCAGTAATGG AGGCCTCCAG CCTGGGCTCT ATTACATTCT TCTCTACAGC  
1901 TGCCCCATAA CCCGTGGCTT ATCCCTGGCA CGTGGGGCCA CACCCACGC  
1951 CCCCTGGATA GGCAACTG TCCTGCTCCA GCCTGTGCTG ANATGAAGT  
2001 TACTCCTAAT TTTTTTTTAA AAAAAAAGTA TTAAATNTCT CTTTCTATAT  
2051 AAAANAAAGN TGGCCCTANN NGGA

**FIG. 32**  
**SUBSTITUTE SHEET (RULE 26)**

1 CCTCACTCGC TCTCCCCGCG CACGCTCCGT CTCCGTCAGT CCCCTGAGCT  
51 GTTCTAGTGC GCGGCGTGGA GCCAGGGCTC AGGCTGGTGG AGCGGCCGGG  
101 GCTGGAGGCT GGGAGTGCGG CGCGCACGGC CTCCCCGCGC CATTATCCGC  
151 GCTCGCTTCG GGCGAGGCCG GCGCCAGGAT GGCAGAGATG GGGAGCAAGG  
201 GGGTGACGGC GGGGAAGATC GCCAGCAACG TACAGAAGAA GCTGACCCGA  
251 GCGCAGGAGA AGGTCCTGCA GAAACTGGGG AAGGCGGACG AGACGAAGGA  
301 CGAGCAGTTT GAGCAGTGTG TCCAGAACTT CAATAAGCAG CTGACAGAGG  
351 GTACCCGGCT GCAGAAGGAT CTTGCGACCT ATCTGGCTTC TGTTAAAGCG  
401 ATGCACGAAG CCTCCAAGAA GCTGAGTGAG TGTCTTCAGG AGGTGTACGA  
451 GCCCGAGTGG CCTGGCAGGG ATGAAGCAAA CAAGATTGCA GAGAACAATG  
501 ACCTACTCTG GATGGACTAC CACCAGAAGC TGGTGGACCA GGCTCTGCTG  
551 ACCATGGACA CCTACCTAGG CCAGTTCCCT GATATCAAGT CGCGCATTGC  
601 CAAGCGGGGG CGGAAGCTGG TGGACTATGA CAGTGCCCGG CACCACTATG  
651 AGTCTCTTCA AACCGCCAAA AAGAAGGATG AAGCCAAAAT TGCCAAGGCA  
701 GAAGAGGAGC TCATCAAAGC CCAGAAGGTG TTCGAGGAGA TGAACGTGGA  
751 TCTGCAGGAG GAGCTGCCAT CCCTGTGGAA CAGCCGTGTA GGTTTCTATG  
801 TCAACACGTT CCAGAGCATC GCGGGTCTGG AGGAAAACCT CCATAAAGAG  
851 ATGAGTAAGC TCAATCAGAA CCTCAATGAT GTCCTGGTCA GCCTAGAGAA  
901 GCAGCACGGG AGCAACACCT TCACAGTCAA GGCCCAACCC AGTGACAATG  
951 CCCCTGAGAA AGGGAACAAG AGCCCGTCAC CTCCTCCAGA TGGCTCCCCT  
1001 GCTGCTACCC CTGAGATCAG AGTGAACCAT GAGCCAGAGC CGGCCAGTGG  
1051 GGCCTCACCC GGGGCTACCA TCCCAAGTC CCCATCTCAG CCAGCAGAGG  
1101 CCTCCGAGGT GGTGGGTGGA GCCCAGGAGC CAGGGGAGAC AGCAGCCAGT  
1151 GAAGCAACCT CCAGCTCTCT TCCGGCTGTG GTGGTGGAGA CCTTCTCCGC  
1201 AACTGTGAAT GGGGCGGTGG AGGGCAGCGC TGGGACTGGA CGCTTGGACC  
1251 TGCCCCCGGG ATTCATGTTC AAGGTTCAAG CCCAGCATGA TTACACGGCC  
1301 ACTGACACTG ATGAGCTGCA ACTCAAAGCT GCGGATGTGG TGTTGGTGAT  
1351 TCCTTTCCAG AACCCAGAGG AGCAGGATGA AGGCTGGCTC ATGGGTGTGA  
1401 AGGAGAGCGA CTGGAATCAG CACAAGGAAC TGGAGAAATG CCGCGGCGTC  
1451 TTCCCGGAGA ATTTTACAGA GCGGCTACAG TGACGGAGGA GCCTTCCGGA  
1501 GTGTGAAGAA CCTTTCCCCC AAAGATGTGT G

FIG. 34

SUBSTITUTE SHEET (RULE 26)

34/61

1 GAATTCGTCG ACCCACGCGT CCGGTTTGAG CAGTGCGTCC  
41 AGAATTTCAA CAAGCAGCTG ACGGAGGGCA CCCGGCTGCA  
81 GAAGGATCTC CGGACCTACC TGGCCTCCGT CAAAGCCATG  
121 CACGAGGCTT CCAAGAAGCT GAATGAGTGT CTGCAGGAGG  
161 TGTATGAGCC CGATTGGCCC GGCAGGGATG AGGCAAACAA  
201 GATCGCAGAG AACAACGACC TGCTGTGGAT GGATTACCAC  
241 CAGAAGCTGG TGGACCAGGC GCTGCTGACC ATGGACACGT  
281 ACCTGGGCCA GTTCCCCGAC ATCAAGTCAC GCATTGCCAA  
321 GCGGGGGCGC AAGCTGGTGG ACTACGACAG TGCCCGGCAC  
361 CACTACGAGT CCCTTCAAAC TGCCAAAAG AAGGATGAAG  
401 CCAAAATTGC CAAGGCCGAG GAGGAGCTCA TCAAAGCCCA  
441 GAAGGTGTTT GAGGAGATGA ATGTGGATCT GCAGGAGGAG  
481 CTGCCGTCCC TGTGGAACAG CCGCGTAGGT TTCTACGTCA  
521 ACACGTTCCA GAGCATCGCG GGCCTGGAGG AAAACTTCCA  
561 CAAGGAGATG AGCAAGCTCA ACCAGAACCT CAATGATGTG  
601 CTGGTCGGCC TGGAGAAGCA ACACGGGAGC AACACCTCCA  
641 CGGTCAAGGC CCAGCCCAGT GACAACGCGC CTGCAAAAGG  
681 GAACAAGAGC CCTTCGCCTC CAGATGGCTC CCCTGCCGCC  
721 ACCCCCGAGA TCAGAGTCAA CCACGAGCCA GAGCCGGCCG  
761 GCGGGGCCAC GCCCGGGGCC ACCCTCCCCA AGTCCCCATC  
801 TCAGCCAGCA GAGGCCTCGG AGGTGGCGGG TGGGACCCAA  
841 CCTGCGGCTG GAGCCCAGGA GCCAGGGGAG ACGCGGGCAA  
881 GTGAAGCAGC CTCCAGCTCT CTTCTGCTG TCGTGGTGA  
921 GACCTTCCA GCAACTGTGA ATGGCACCGT GGAGGGCGGC  
961 AGTGGGGCCG GCGCTTGGA CCTGCCCCA GGTTCATGT  
1001 TCAAGGTACA GGCCAGCAC GACTACACGG CCACTGACAC  
1041 AGACGAGCTG CAGCTCAAGG CTGGTGATGT GGTGCTGGTG  
1081 ATCCCCTTCC AGAACCCTGA AGAGCAGGAT GAAGGCTGGC  
1121 TCATGGGCGT GAAGGAGAGC GACTGGAACC AGCACAAGGA  
1161 GCTGGAGAAG TGCCGTGGCG TCTTCCCCGA GAACTTCACT  
1201 GAGAGGGTCC CATGACGGCG GGGCCCAGGC AGCCTCCGGG  
1241 CGTGTGAAGA ACACCTCCTC CCGAAAAATG TGTGGTTCTT  
1281 TTTTTTGTTT TGTTTTCGTT TTTTCATCTT TGAAGAGCAA  
1321 AGGGAAATCA AGAGGAGACC CCCAGGCAGA GGGGCGTTCT  
1361 CCCAAAGATT AGGTCGTTTT CCAAAGAGCC GCGTCCCGGC  
1401 AAGTCCGGCG GAATTCACCA GTGTCCTGAA GCTGCTGTGT  
1441 CCTCTAGTTG AGTTCTGGCG CCCCTGCCTG TGCCCGCATG  
1481 TGTGCCTGGC CGCAGGGCGG GGCTGGGGG TGCCGAGCCA  
1521 CCATGCTTGC CTGAAGCTTC GGCCGCGCCA CCCGGGCAAG  
1561 GGTCCTCTTT TCCTGGCAGC TGCTGTGGGT GGGGCCAGA  
1601 CACCAGCCTA ACCTGGCTCT GCCCCGAGA CGGTCTGTGT  
1641 GCTGTTTGAA AATAAATCTT AGTGTTCAAA ACAAATGAA  
1681 ACAAAAAAAA TGATAAAAAA AAAAAAAA AAAAAAAA  
1721 AAAAGGGCGG CCGC

**FIG. 36**  
SUBSTITUTE SHEET (RULE 26)

35/61

1 EFVDPRVRFE QCVQNFNKQL TEGTRLQKDL RTYLASVKAM  
 41 HEASKKLNEC LQEVYEPDWP GRDEANKIAE NNDLLWMDYH  
 81 QKLVDQALLT MDTYLGQFPD IKSRIAKRGR KLVDYDSARH  
 121 HYESLQTAKK KDEAKIAKAE EELIKAQKVF EEMNVDLQEE  
 161 LPSLWNSRVG FYVNTFQSIA GLEENFHKEM SKLNQNLNDV  
 201 LVGLEKQHGS NTSTVKAQPS DNAPAKGNKS PSPPDGSPAA  
 241 TPEIRVNHEP EPAGGATPGA TLPKSPSQPA EASEVAGGTQ  
 281 PAAGAQEPGE TAASEAASSS LPAVVVETFP ATVNGTVEGG  
 321 SGAGRLDLPP GFMFKVQAQH DYTATDTDEL QLKAGDVVLV  
 361 IPFQNPEEQD EGWLMGVKES DWNQHKELEK CRGVFPENFT  
 401 ERVP

## FIG. 37

1 MWKSVVGHDV SVSVETQGDD WTDPDFVND ISEKEQRWGA KTIEGSGRTE  
 51 HINIHQLRNK VSEHDILKK KELESGPKAS HGYGGQFGVE RDRMDKSAVG  
 101 HEYVADVEKH SSQTDAARGF GGKYGVERDR ADKSAVGFDY KGEVEKHASQ  
 151 KDYSHGFGGR YGVEKDKRDK AALGYDYKGE TEKHESQRDY AKGFGGQYGI  
 201 QKDRVDKSAV GFNEMEAPTT AYKKTPIEA ASSGARGLKA KFESLAEKRR  
 251 KREEEKAQQA MARQQERKA VVKMSREVQQ PSMPVEEPAA PAQLPKKISS  
 301 EVWPPAESHL PPESQPVRSR REYPVPSLPT RQSPLGNHLE DNEEPPALPP  
 351 RTPEGLQVVE EPVYEAAPEL EPEPEPDYEP EPETEPDYED VGELDRQDED  
 401 AEGDYEDVLE PEDTPSLSYQ AGPSAGAGGA GISAIALYDY QGEGSDELSF  
 451 DPDDIITDIE MVDEGWWRGQ CRGHFGLFPA NYVKLL

## FIG. 39

1 MAGNFDSEER SSWYWGRLSR QEAVALLQGO RHGVFLVRDS STSPGDYVLS  
 51 VSENSRVSHY IINSSGPRPP VPPSPAQPPP GVSPSRLRIG DQEFDSLAL  
 101 LEFYKIHLYD TTTLIEPVAR SRQSGVILR QEEAEYVRAL FDFNGNDEED  
 151 LPFKKGDILR IRDKPEEQWW NAEDSEGKRG MIPVPYVEKY RPASASVSAL  
 201 IGGNQEGSHP QPLGGPEPGP YAQPSVNTPL PNLQNGPIYA RVIQKRVNA  
 251 YDKTALALEV GELVKVTKIN VSGQWEGECN GKRGHFPFTH VRLLDQQNPD  
 301 EDFS

## FIG. 41

SUBSTITUTE SHEET (RULE 26)



36/61

1 CAGCCGCTGG AGGGGGCGCC TGGTGTAGAT GTGAAAAGCC GTAACCAGGA  
51 ACCAGTAAAG ATGTGGAAGT CTGTAGTGGG GCATGATGTA TCGGTTTCCG  
101 TGGAGACCCA GGGTGATGAC TGGGATACAG ACCCTGACTT TGTGAATGAC  
151 ATCTCCGAGA AGGAGCAACG GTGGGGAGCC AAGACCATTG AGGGCTCTGG  
201 ACGCACAGAG CACATCAACA TCCACCAGCT GAGGAACAAA GTGTCAGAGG  
251 AGCACGACAT CCTCAAGAAG AAGGAGCTGG AATCGGGGCC TAAGGCATCC  
301 CATGGCTATG GCGGTCAGTT TGGAGTGGAG AGAGACCGGA TGGACAAGAG  
351 TGCCGTGGGC CACGAGTATG TTGCTGATGT GGAGAAACAC TCATCTCAGA  
401 CTGATGCSGC CAGAGGCTTT GGGGGCAAAT ATGGAGTTGA GAGGGACCGG  
451 GCAGACAAGT CAGCGGTGGG CTTTGACTAC AAAGGAGAAG TGGAAAAGCA  
501 TGCATCTCAG AAAGATTACT CTCATGGCTT TGGTGGCCGC TACGGGGTAG  
551 AGAAGGATAA ACGGGACAAA GCAGCCCTGG GATACGACTA CAAAGGAGAG  
601 ACGGAGAAGC ACGAGTCTCA GAGAGATTAT GCCAAGGGCT TTGGTGGCCA  
651 ATATGGAATC CAGAAAGACC GAGTGGATAA GAGTGCTGTT GGCTTCAATG  
701 AAATGGAGGC CCAACCACG GCGTATAAGA AGACAACACC CATAGAAGCT  
751 GCTTCCAGTG GTGCCCCTGG GCTGAAGGCA AAATTTGAGT CCCTGGCTGA  
801 GGAGAAGAGG AAGCGAGAGG AAGAAGAGAA GGCACAGCAG ATGGCCAGGC  
851 AGCAACAGGA GCGAAAGGCT GTGGTAAAGA TGAGCCGAGA AGTCCAGCAG  
901 CCATCCATGC CTGTGGAAGA GCCAGCGGCA CCAGCCCAGT TGCCCAAGAA  
951 GATCTCCTCA GAGGTCTGGC CTCCAGCAGA GAGTCACCTA CCGCCAGAGT  
1001 CTCAGCCAGT GAGAAGCAGA AGGGAATACC CTGTGCCCTC TCTGCCACG  
1051 AGGCAGTCTC CATTGCAGAA TCACTTGGAG GACAACGAGG AGCCCCCAGC  
1101 TCTGCCCCCT AGGACCCCAG AAGGCCTCCA GGTGGTGGAA GAGCCAGTGT  
1151 ACGAAGCAGC ACCCGAGCTG GAGCCGGAGC CAGAGCCTGA CTATGAGCCA  
1201 GAGCCAGAGA CAGAGCCTGA CTATGAGGAT GTTGGGGAGT TAGATCGGCA  
1251 GGATGAGGAT GCAGAGGGAG ACTATGAGGA TGTGCTGGAG CCCGANGACA  
1301 CCCCTTCTCT GTCCTACCAA GCTGGACCCT CAGCTGGGGC TGGTGGTGCG  
1351 GGGATCTCTG CTATAGCCCT GTATGATTAC CAAGGAGAGG GAAGCGATGA  
1401 GCTTTCCTTT GATCCAGATG ACATCATCAC TGACATTGAG ATGGTGGATG  
1451 AAGGCTGGTG GCGGGGCCAA TGCCGTGGCC ACTTTGGACT TTTCCCTGCA  
1501 AACTATGTCA AGCTCCTCTA ATGACCAGCC CATTGTCTTC CGACTTCCCG  
1551 AATTCGAAGC TGCTCTGCCT CCCTCTTCCC ACTCCATGGT ACTGCTGCAA  
1601 GGACCTGGCT GAACATCATG AGATGCCTGA AGTTCTGGCA GTCTGTCTCC  
1651 CGCCTCTTTA AGAGCTTTAG GTAGAATCGC TCCAGGTGGG GGTGGGGGTG  
1701 GGGGTGGGAT CCCTCTGTCC CTCTGTGACC ACTCTTCCCT GAGGTAGCTC  
1751 ATGAAATCAT CTTGCAGACC TGCCTCCTTC AGCCGCACCC CAGCTCTGCC  
1801 AACCTTGCTC TAGAGTGCTG GGATTCCCTT GCCCGACCC TGGGTGCCAG  
1851 CCTAGAGGGG AGGCTCTCAC AGGGCTGCCT GATTCGCCCT GTTGTGCTTT  
1901 TGCTCATTTT TCTTCCCTTA GCAGACAAAT TGGAACTGCC CTTCTGTTTA  
1950 GTCCTAAAC TGAAAATAAA ATGAGACTGT GGCTAAAAAA AAAAAAAAAA  
2003 AAA

FIG. 38  
SUBSTITUTE SHEET (RULE 26)

## 37/61

1 GGATCCCCGG AGCCGGTCCG CTGGGCGGGG CGCAGGGCTG GAGGGGCGCG  
 51 CGTGCCGGCG GCGGCCAGC GTGAAAGCGC GGAGGCGGCC ATGGCGGGCA  
 101 ACTTCGACTC GGAGGAGCGG AGTAGCTGGT ACTGGGGCCG CCTGAGCCGG  
 151 CAGGAGGCGG TGGCGCTATT GCAGGGCCAG CGGCACGGGG TGTTCCTGGT  
 201 GCGGGACTCG AGCACCAGCC CCGGGGACTA TGTGCTTAGC GTCTCCGAAA  
 251 ACTCGCGCGT CTCCCACTAC ATCATCAACA GCAGCGGCCC GCGCCCTCCA  
 301 GTGCCTCCGT CGCCCGCTCA GCCTCCGCCG GGAGTGAGTC CCTCCAGGCT  
 351 CCGAATAGGA GATCAAGAAT TTGATTCAAT GCCTGCTTTA CTGGAATTCT  
 401 ACAAATACA CTATTTGGAC ACTACAACAT TGATAGAACC AGTGGCCAGA  
 451 TCAAGGCAGG GTAGTGGAGT GATTCTCAGG CAGGAGGAGG CAGAGTATGT  
 501 GCGGGCCCTG TTTGACTTTA ATGGGAATGA TGAAGAAGAT CTTCCCTTTA  
 551 AGAAAGGAGA CATCCTGAGA ATCCGGGATA AGCCTGAAGA CGAGTGGTGG  
 601 AATGCAGAGG ACAGCGAAGG AAAGAGGGGG ATGATTCCTG TCCCTTACGT  
 651 GGAGAAAGTAT AGACCTGCCT CCGCCTCAGT ATCGGCTCTG ATTGGAGGTA  
 701 ACCAGGAGGG TTCCACCCA CAGCCACTGG GTGGGCCGGA GCCTGGGCCC  
 751 TATGCCCAAC CCAGCGTCAA CACTCCGCTC CCTAACCTCC AGAATGGGCC  
 801 CATTTATGCC AGGGTTATCC AGAAGCGAGT CCCTAATGCC TACGACAAGA  
 851 CAGCCTTGGC TTTGGAGGTC GGTGAGCTGG TAAAGGTTAC GAAGATTAAT  
 901 GTGAGTGGTC AGTGGGAAGG GGAGTGTAAT GGCAAACGAG GTCATTCCC  
 951 ATTCACACAT GTCCGTCTGC TGGATCAACA GAATCCCGAT GAGGACTTCA  
 1001 GCTGAGTATA GCTCGACAGT TTGCTGACAG ATGGAACAAT CTGTTTTCCC  
 1051 CCAATTGCCA TCTATACAAT TTTCTTACAG GTGTCAAAGC AGTCTAGTTT  
 1101 ATATAAGCAT TCTGTTACCT GGGATCTTTT TTAAGACTGA ACTACTCCAT  
 1151 TCTCACTTGT ATTTACCATA TTCAGGGTAC GAAACCGGAG GGCTTATGTG  
 1201 GTTAACTTCT GAGTTGGCAG TTTTAGGTGG TAGTGGCCGT GCCTGTATGA  
 1251 GAAGACGTAA ATACATTGCC TCCTTTCTTT TGGGCAAAAC AGATCA

## FIG. 40

1 MSSECDVGSS KAVVNGLASG NHGPKDMDP TKICTGKGTV TLRASSSYRG  
 51 TPSSSPVSPQ ESPKHESKSD EWKLSSSADT NGNAQPSPLA AKGYRSVHPS  
 101 LSADKPQGSF LLNEVSSSHI ETDSQDFPPT SRPSSAYPST TIVNPTIVLL  
 151 QHNREQQKRL SSDLSPASER RAGEQDPVPT PAELTSPGRA SERRAKDASR  
 201 RVVRSADLS DVSTDEVGIP LRNTERSKDW YKTMFKQIHK LNRDDSDSVH  
 251 SPRYSFSDDT KSPLSVPRSK SEMNYIEGK VVKRSATLPL PARSSSLKSS  
 301 PERNDWEPLD KKVDTKRYA EPKSIYEYQP GKSSVLTNEK MSRDISPEEI  
 351 DLKNEPWYKF FSELEFGRPS SAVSPTPDIT SEPPGYIYSS NFHAVKRESO  
 401 GTPGGLASLE NERQIYKSVL EGGDIPLQGL SGLKRPSSSA STKDSSEPRH  
 451 FIPADYLEST EEFIRRRHDD KEKLLADQRR LKREQEADI AARRHTGVIP  
 501 THHQFITNER FGDLLNIDDT AKRKSGLEMR PARAKFDFKA QTLKELPLQK  
 551 GDVVYIYRQI DQNWYEGEHH GRVGIFPTY IELLPPAEKA QPRKLAPVQV  
 601 LEYGEAIAKF NFNGDTQVEM SFRKGERITL LRQVDENWYE GRIPGTSRQG  
 651 IFPITYVDVL KRPLVKTPVD YIDLPSYSSP SRSATVSPQA SHHLSAGPD  
 701 LTESSEKNYVQ PQAQORRVTP DRSQPSLDLC SYQALYSYVP QNDDELELRD  
 751 GDIVDMEKC DDGWFVGTSR RTRQFGTFPG NYVKPLYL

## FIG. 43

## SUBSTITUTE SHEET (RULE 26)

38/61

1 CCTCACCGNN CCTGGTGTAG GTACCGGATC GAATTCAAGC GAAAAACAGA  
 51 GCGGGGCTGA CTGTAGCGTG GAGCGCGAGC CGGGCTGGAC GCGCGCAAGC  
 101 CCTTGCCGGG GACCCGCGAG GCAAGCAGTC TCCCTGTGGA GCGTCGTCTT  
 151 CCATCCCTGT AAGCACCGTT ACAGAGAATG AAACAAGGGC AGAAGTTACA  
 201 GAGCCCGTGA GGCATCTTCA AATAGAAGAC TGGAGACTAG AAASAGAATA  
 251 TTGCCAGGAG TTGGCATCCA TTGGAAGACC TTGAGATCCT CTCAGCTCAC  
 301 AACTCCAGGA CCGATGCATC TTCCCACCAC CTTGAAGCAC TGAGCCCTCC  
 351 AGAGCTGCAT CTGGGAAGAC TCGCCTGCCT CCAGCATGAG TTCTGAATGT  
 401 GATGTTGGAA GCTCTAAAGC TGTGGTGAAT GGCTTGGCAT CTGGCAACCA  
 451 TGGACCAGAC AAAGACATGG ACCCTACCAA AATCTGCACT GGGAAAGGAA  
 501 CAGTGACTCT TCGGGCCTCG TCTTCTTACA GGGGAACCCC AAGCAGCAGC  
 551 CCTGTGAGCC CCCAGGAATC TCCGAAGCAT GAAAGCAAGT CAGATGAATG  
 601 GAAACTTTCT TCCAGTGCAG ATACCAATGG CAACGCCAG CCCTCCCCAC  
 651 TTGCTGCCAA GGGCTATAGA AGTGTGCATC CCAGCCTTTC TGCTGACAAG  
 701 CCCCAGGGCA GTCCTTTACT AAACGAAGTT TCTTCTTCCC ACATTGAAAC  
 751 CGATTCCCAA GACTTCCCTC CAACAAGCAG ACCTTCGTCT GCCTACCCCT  
 801 CCACCACCAT CGTCAACCCT ACCATTGTGC TCCTGCAGCA CAATCGAGAG  
 851 CAGCAAAAGC GACTCAGTAG TCTTTCAGAT CCTGCCTCAG AGAGAAGAGC  
 901 GGGTGAGCAG GACCCAGTAC CAACCCAGC AGAACTCACT TCGCCCGGCA  
 951 GGGCTTCTGA GAGAAGGGCA AAGGATGCTA GCAGACGGGT GGTGAGGAGC  
 1001 GCACAGGACC TGAGCGATGT GTCTACAGAT GAAGTGGGCA TTCCACTCCG  
 1051 GAATACCGAG CGATCGAAAG ACTGGTACAA AACTATGTTT AAACAGATCC  
 1101 ACAAACTGAA CAGAGATGAT GATTCTGATG TCCATTCCCC TCGATACTCC  
 1151 TTCTCTGATG ACACAAAGTC TCCCCTTTCT GTGCCTCGCT CAAAAAGTGA  
 1201 GATGAACTAC ATCGAAGGGG AGAAAGTGGT TAAGAGGTCC GCCACACTCC  
 1251 CCCTCCCAGC CCGCTCTTCC TCACTCAAGT CCAGCCCGGA AAGAAACGAC  
 1301 TGGGAGCCCC TAGATAAGAA AGTGGATACG AGAAAATACC GAGCAGAGCC  
 1351 CAAAAGCATT TACGAATATC AGCCGGGCAA GTCTTCGGTC CTGACCAATG  
 1401 AGAAGATGAG TCGGGATATA AGCCCAGAAG AGATAGATT TAAAGAATGAA  
 1451 CTTGGTATA AATTCTTTTC GGAATTGGAG TTTGGGAGAC CGAGCTCAGC  
 1501 AGTCAGCCCC ACTCCAGACA TTACGTCAGA GCCTCCTGGA TATATCTATT  
 1551 CTTCCAACCT CCATGCAGTG AAGAGAGAAT CGGACGGGAC CCCCAGGGGT  
 1601 CTCGCTAGCT TGGAGAATGA GAGGCAGATC TATAAGAGTG TCTTGAAGC  
 1651 TGGCGACATC CCTCTTCAGG GCCTCAGTGG GCTCAAGCGA CCTTCCAGCT  
 1701 CAGCTTCCAC TAAAGATTCA GAGTCACCAA GACATTTTAT ACCAGCTGAT  
 1751 TACTTGGAGT CCACAGAAGA ATTTATTCGG AGACGGCACG ATGATAAAGA  
 1801 GAAACTTTTA GCGGACCAGA GACGACTTAA GCGCGAGCAA GAAGAGGCCG  
 1851 ATATTGCAGC TCGCCGCCAC ACAGGTGTCA TCCCGACTCA TCATCAGTTT  
 1901 ATCACTAATG AGCGCTTTGG GGACCTCCTC AATATAGATG ATACGGCCAA  
 1951 AAGGAAATCT GGGTTAGAGA TGAGACCTGC TCGAGCCAAA TTTGACTTTA  
 2001 AAGCCAGAC CCTGAAGGAG CTGCCTCTGC AGAAGGGAGA CGTTGTTTAC  
 2051 ATCTACAGAC AGATTGACCA GAACTGGTAT GAAGGTGAAC ACCATGGCCG  
 2101 GGTGGGAATC TTCCCACGCA CCTATATCGA GCTTCTTCT CCAGCTGAGA  
 2151 AGGCTCAGCC CAGAAAGTTG GCACCCGTAC AAGTTTTGGA ATATGGAGAA

FIG. 42A

SUBSTITUTE SHEET (RULE 26)

39/61

2201 GCCATTGCAA AGTTTAACTT TAATGGAGAT ACACAAGTAG AAATGTCTTT  
2251 CCGAAAGGGG GAGAGGATCA CGCTGCTCCG ACAGGTGGAT GAGAACTGGT  
2301 ATGAAGGGAG GATTCCTGGG ACATCTCGCC AAGGCATTTT CCCTATCACC  
2351 TATGTAGATG TGCTTAAGAG GCCATTGGTG AAAACCCCTG TGGATTACAT  
2401 CGACCTGCCT TATTCTTCTT CCCCAAGTCG CAGTGCCACT GTGAGCCAC  
2451 AGGCTTCTCA TCATTCATTG AGCGCAGGAC CTGATCTCAC AGAATCTGAA  
2501 AAGAACTATG TGCAACCTCA AGCCCAGCAG CGAAGAGTCA CCCCAGACAG  
2551 GAGTCAGCCC TACTGGATT TGTGTAGCTA CCAAGCGTTA TATAGTTATG  
2601 TGCCACAGAA CGATGATGAG TTGGAAGTCC GAGATGGAGA TATTGTTGAT  
2651 GTCATGGAAA AATGTGACGA TGGATGGTTT GTTGGCACTT CGAGAAGGAC  
2701 GAGGCAGTTT GGTACTTTTC CAGGCAACTA TGTAAAACCT TTATATCTAT  
2751 AAGAAGACTA AAAAGCACAG AGATTATTTT TTATCGGAGG ATGAAGCATC  
2801 ATTCATGAAC TGGTCTCTTT ATTTAAGTAC TGAGTCAGTA AGAAAACTAA  
2851 TGCAGTTGGT AAAGAAAGAA TTCAAAGAAG GAACAGAGAA GTGTGTTTGA  
2901 AACCCATTGT GTATCAGGGA TTAAGTATCT GCTGAAGACA TCTGTATTTA  
2951 CATGACTGCT TCTGGGAGCT GCTCTAGCCC CCGCTGCTTG GGGAACTCTGA  
3001 TCTGGAGCAT GTCCATGAGC AACATTAGCC AAAAAAAAAA GCTTGGGCCC  
3051 TATTCTATAG TGTCACCTAA ATACTAGCTT GATCCGGCTG CTAACAAAGC  
3101 CCGAAAGGAA GCTGAGTTGC TGCTGCCACC GCTGAGCAAT AACTAGCATA  
3151 ACCCCTTGGG GCCTCTAAAC GGGTCTTGAG GGGTTTTTTG GCTGAAAGGA  
3201 GGAAGTATAT CCGGATAACC TGGCGTAATA GCGAAGAGGC CCGCACCGAT  
3251 CGCCCTTCCC AACAGTTGGG CAGCCTGAAT GGCGAATGGA CGCGCCCTGT  
3301 AGCGGCGCAT TAAGCGCGGC GGGTGTGGTG GTTACGCGCA GGGTG

FIG. 42B

SUBSTITUTE SHEET (RULE 26)

40/61

1 TTNNCACTCA CCGTCCTGGT GATGGTACCG GATCGAATTC AAGCGTGGCC  
51 GTGGCCGTGG GGCGCGCGGG GACCGCCCGG GGTGCCCCT CCGCTCAGCG  
101 TCCGGGCCGC GTGGTCCGGC GGAGCCCCGA GACCACCCCC GGGCGGGGCG  
151 CCGCCGCGAT GTCGGTGGCT GGGCTCAAGA AGCAGTTCCA CAAAGCCAGC  
201 CAGCTGTTTA GTGAAAAAAT AAGTGGTGCC GAAGGAACGA AGCTAGATGA  
251 AGAATTTCTG AACATGGAAA AGAAAATAGA TATCACCAGT AAAGCTGTTG  
301 CAGAAATCCT TTCAAAGGCC ACAGAGTATC TCCAACCCAA TCCAGCATAC  
351 AGAGCTAAGC TAGGAATGCT GAACACTGTG TCGAAGCTCC GAGGGCAGGT  
401 GAAGGCCACC GGCTACCCAC AGACGGAAGG CTTGCTGGGG GACTGCATGC  
451 TGAAGTATGG CAAGGAGCTC GGAGAAGACT CTGCTTTTGG CAACTCGTTG  
501 GTAGATGTTG GTGAGGCCCT GAAACTCATG GCTGAGGTGA AAGACTCTCT  
551 GGATATTAAT GTGAAGCAA CTTTTATTGA CCCACTGCAG CTACTGCAAG  
601 ACAAAGATTT AAAGGAGATC GGGCACCACC TGAGAAAGCT GGAAGGCCGT  
651 CGCCTGGATT ATGATTATAA AAAGCGGCGG GTAGGTAAGA TCCCCGAGGA  
701 AGAAATCAGA CAAGCAGTAG AGAAGTTTGA AGAGTCAAAG GAGTTGGCCG  
751 AAAGGAGCAT GTTTAATTTT TTAGAAAATG ATGTAGAGCA AGTGAGCCAG  
801 CTGGCTGTGT TTGTAGAGGC GGCATTAGAC TATCACAGGC AGTCCACAGA  
851 GATCCTCCAG GAGCTGCAGA GCAAGCTGGA GTTGCGAATA TCTCTTGCAT  
901 CCAAAGTCCC CAAGCGAGAA TTCATGCCAA AGCCTGTGAA CATGAGTTCC  
951 ACCGATGCCA ATGGGGTCGG ACCCAGCTCT TCATCAAAGA CACCAGGTAC  
1001 TGACACTCCC GCGGACCAGC CCTGCTGTCG TGGTCTCTAT GACTTTGAGC  
1051 CAGAAAATGA AGGAGAATTA GGATTTAAAG AAGGGGACAT CATTACATTA  
1101 ACCAATCAGA TAGATGAAAA CTGGTATGAA GGGATGCTTC GTGGGGAATC  
1151 CGGCTTCTTC CCCATTAATT ACGTGGAAGT CATTGTGCCT TTACCTCCGT  
1201 AAATGTGTCT TTTGGACCTA ACTTCAGAAC TGAAATGAAT TGGCACCAGT  
1251 GCTCTCTCAG TGTGGTGTTT TGTGACANCC TCGCTCTCTG GCCCACTTAA  
1301 TCACTTTTGT ATGTGTGTTT TCTTTATAAT GTATTTTGTA TCAATTTAAT  
1351 TTGTATAACT GATTTCTTTG TCCTAACTCA TAAAAATAGT TTTCTTCTTG  
1401 TTCTAAAAAG TCATTGGTTA AATGTATTTG CTTCTGTGTG CTAAAACGAG  
1451 TAAATTGCGC CCATTGCAAT GGCCTGGGTA GTCCTTGACT GCAGTGGGAA  
1501 CGCACCCCTT GCAGCCATGA AAGCTAAAGG TTTGTTTCCT GACATTATTG  
1551 ATGGCCTCTG GTCTTTTCCT GTTTTAAGCT TACCTGTGAA CAGCCCAATA  
1601 AACNTGACAC ACTGTANAAT AANAAGGGTG GCCCNA

FIG. 44

SUBSTITUTE SHEET (RULE 26)

41/61

1 MSVAGLKKQF HKASQLFSEK ISGAEGTKLD EEFLNMEKKI DITSKAVAEI  
51 LSKATEYLQP NPAYRAKLG M LNTVSKLRGQ VKATGYPQTE GLLGDCMLKY  
101 GKELGEDSAF GNSLVDVGEA LKLMAEVKDS LDINVKQTFI DPLQLLDQKD  
151 LKEIGHHLRK LEGRRLDYDY KKRRVGGIPE EEIRQAVEKF EESKELAERS  
201 MFNLENDVE QVSQLAVFVE AALDYHRQST EILQELQSKL ELRISLASKV  
251 PKREFMPKPV NMSSTDANGV GPSSSSKTPG TDTPADQPCC RGLYDFEPEN  
301 EGELGFKEGD IITLTNQIDE NWYEGMLRGE SGFFPINYVE VIVPLPP

FIG. 45

1 MSGSYDEASE EITDSFWEVG NYKRTVKRID DGHRLCNDLM SCVQERAKIE  
51 KAYAQQLTOW AKRWRQLIEK GPQYGSLEA WGAMMTEADK VSELHQEVKN  
101 SLLNEDLEKV KNWQKDAYHK QIMGGFKETK EAEDGFRKAQ KPWAKKMKEL  
151 EAAKKAYHLA CKEERLAMTR EMNSKTEQSV TPEQQKKLVD KVDKCRQDVQ  
201 KTQEKYEKVL EDVGKTTPOY MEGMEQVFEQ CQQFEEKRLV FLKEVLDDIK  
251 RHLNLAENSS YMHVYRELEQ AIRGADAQED LRWFRSTSGP GMPMNWPOFE  
301 EWNPDLPHTT AKKEKQPKKA EGATLSNATG AVESTSQAGD RGSVSSYDRG  
351 QTYATEWSDD ESGNPFGGNE ANGGANPFED DAKGVRVRAL YDYGQEQDE  
401 LSFKAGDELT KLGEDEQGW CRGRLDGSQL GLYPANYVEA I

FIG. 47

SUBSTITUTE SHEET (RULE 26)

1 CGGGCTTGAG GCTGGGCCGC CGCCGCCGCC CGCTTTGCCA CCCGCCCCGC  
51 TGATGGTGTG CGGTGCTCCG GCGCCAGGG ACACAGACCG GGAGCAGGAC  
101 CACTTCTCTC ACCTCCGGAT CTCTCCTGCT TCCGCAGCCT GTGAGCAGCA  
151 GGCCTGCTAA CTGCAGATCC ACAACCGCAC AGCTCGCTAC AGGTGCACCA  
201 TGTCTGGCTC CTACGATGAG GCCTCAGAGG AGATCACAGA TAGCTTCTGG  
251 GAGGTGGGGA ACTACAAGCG GACGGTGAAG CGCATCGACG ATGGGCAACCG  
301 CCTGTGCAAC GACCTCATGA GCTGCGTGCA GGAGCGCGCC AAGATCGAGA  
351 AGGCATACGC GCAGCAGCTC ACCGACTGGG CCAAGCGCTG GCGCCAGCTC  
401 ATCGAGAAAG GTCCTCAGTA TGGCAGCCTG GAGCGGGCGT GGGGCGCCAT  
451 GATGACAGAA GCAGATAAGG TCAGCGAGCT GCACCAGGAG GTGAAGAACA  
501 GCCTGCTGAA TGAGGACCTG GAGAAAGTCA AGAACTGGCA GAAGGATGCC  
551 TATCACAAGC AGATCATGGG TGGCTTCAAG GAGACGAAAG AGGCCGAGGA  
601 TGGCTTCCGA AAGGCCCAGA AGCCCTGGGC TAAAAAGATG AAGGAGCTAG  
651 AGGCGGCCAA GAAGGCCTAT CACTTGGCTT GTAAGGAGGA AAGGCTGGCC  
701 ATGACCCGGG AGATGAACAG TAAGACAGAG CAGTCGGTCA CCCCTGAACA  
751 GCAGAAAGAA CTTGTGGACA AAGTGGACAA ATGCAGACAG GATGTGCAAA  
801 AGACTCAGGA GAAGTATGAG AAGTCTCTGG AAGATGTGGG CAAGACCACA  
851 CCACAGTACA TGGAGGGCAT GGAGCAGGTG TTTGAGCAGT GCCAGCAGTT  
901 TGAGGAGAAG CGGCTGGTCT TCCTGAAGGA AGTCCTGCTG GATATCAAAC  
951 GGCATCTCAA CCTAGCGGAG AACAGCAGCT ACATGCATGT CTACCGAGAA  
1001 CTGGAGCAGG CCATCCGGGG GGCCGATGCC CAGGAGGACC TCAGGTGGTT  
1051 CCGCAGCACC AGTGGCCCCG GGATGCCCAT GAACTGGCCG CAGTTCGAGG  
1101 AGTGAACCC AGACCTCCCG CACACCACTG CCAAGAAGGA GAAACAGCCT  
1151 AAGAAGGCAG AGGGGGCCAC CCTGAGCAAT GCCACTGGGG CTGTAGAATC  
1201 CACATCCCAG GCTGGGGACC GTGGCAGTGT TAGCAGCTAT GACCGAGGCC  
1251 AAACATATGC CACCGAGTGG TCAGACGATG AGAGCGGAAA CCCCTTCGGG  
1301 GGCAATGAGG CCAATGGTGG CGCCAACCC TTCGAGGATG ATGCCAAGGG  
1351 AGTTCGTGTA CGGGCACTCT ATGACTACGA CGGTCAGGAG CAGGATGAGC  
1401 TCAGCTTCAA GGCCGGAGAT GAGCTACCA AGCTCGGAGA GGAAGACGAA  
1451 CAGGGTTGGT GCCGCGGGCG GCTGGACAGC GGACAGCTGG GCCTCTATCC  
1501 TGCCAACTAC GTTGACGCTA TATAGTACC TTGCCCACCC GACTCCTCTC  
1551 AGTCCTTGTC CACCGCCTTC CACCCTTCCC CTCCCCCTTG CCATAGAGTT  
1601 CCAGACATAT TTTCCCATCA AGCTTTTATT TTTTAAAAG TCAAAACAGA  
1651 AAAAAAAAAA AAAAAAAAAA GAAGAAATAC GAAGAGACAG CGTTTGACGC  
1701 CTACCTGGAG GCCGGGGGGG AGGGGGCTTA GGGTGATGGC CTCCCCACA  
1751 GCGTGGGCAA GGATCTTGGG ACTAACCCAA TGTCACATCT GGTCTATAGA  
1801 GTCCACCAAA GAGTCTCCTG AGTCTTGAGG GAGATCTTCT GGATCCTTCT  
1851 ACCCTGTCTC GCTCTCCTAT CCCACCACAG CTGCCAGCAG CTGCCATGT  
1901 CACCTGAGCC TGGCTTCCTA AACTCTCCTG TCCCCTCTCC TGTCCCCCTT  
1951 CAACGCCCCC TTCTCTTAAA GGGCCCCCAA TCTTTAGTCT TCCACTCTGC  
2001 CCTGGGGGTG CTTTTCTCTT CCCAGCCCTG TCCAGTGAGG CTGGGGGAGA  
2051 AGGCTGCGGA GGGGAGGGGA GTGTCTCTTC ACTCCCCAG ACATGAAGGC  
2101 AGGTGAGTGG GAGGGAGTCA TGGCCTCCCT GGCATACAGG AGAGGAAGAA  
2151 GGAGAACAGA CCATCTGACC AGGCTGTGCA AACTCCCAA TGCCAAGCCC

**FIG. 46A**  
**SUBSTITUTE SHEET (RULE 26)**

43/61

2201 ATTTGAGGGA TGAAAACCCT AGCTGGGCCT GTGGGCAGAG GGCTCCTCCT  
2251 CAGAGCCAAT GAGCATTTCG AGAGACCCTA CCTGTCTCTT TAGTCCTTGG  
2301 CAATGGGCAA AGCCTCTTCC TTGGAAAGTC CAGGGCAAAG CCAGCAACAG  
2351 TAGCAACCTC CTCTCACTCT GGGGAGGAGG CATTGGCCAC CCATCCCCCT  
2401 CCCTTCATGG TCATTCAGAA ACGCCACAGC CCCTCCCATC CCCAATCACT  
2451 GTGTCAGCAT CAGCCTTTGT GAAGACGGTC TACAAGGCTC TCACCTGGCC  
2501 AACCTAGGAG ATTCAGGGGC TCAGGAACCT AGGAGATTCA GGGGCTTGGG  
2551 GAACCTCCAC CTTGGCACTG TAAGGGGAAG CCAGCAGCTC AGGCTGGTGT  
2601 GAGGAAGGAA CTCTGGATGG TCACTGTAGC TTTCTTCCTT GACCTTTTAG  
2651 TCCCCAACAT CCCCTCTGAA TGCTGGCAGC ACCCCCACCC CCACACACAC  
2701 ACTCCCATTT CTCTAAGCCC GAGAGTCTTG AGTCTTCATT AAAGGATTCT  
2751 GGGTGTGGGA GGGGACACAG GGCCTTGTGG TTGGGAAGCA GGTGGCAGGC  
2801 TCTCCCTTGG GAGGATGGGG TGGGAAACGA AACAGGTCAA CCAAGACCTC  
2851 TTACAGTGGA AAGTGGTCAG AGGCTGTTTC TTTGGACCTT TGGGAACACA  
2901 GATTTGAGAA AGTCTCATAT TCACAGCTGG TGTCCGCTAG GCCTCTGGCC  
2951 TACGGACACC CTCTGCCTTG TGAATCAGGT GACCTTTTGG GCCTCCAGGG  
3001 AAAGAACAGG ACCACCATCC ATGTTCTCCG CGTCCCTTTA GCTCTCTGCT  
3051 GCTTCTCCTG ACACTCAGGT CATGGACCCA AGCTTTGGGG TCCTGACCAC  
3101 CGCCCCCCCC CACCCCCTT CTCTTGACTA GGCTGCAGCA GGGCCTTCTG  
3151 TTGGGTCACT CCTCCTCAGG GCCAGGAGCA GGAACCTAGC ACTCAAGAGA  
3201 CAGGGCTGTA AGCACCCACT TCCCTGTCAC TGTTTGCCCT TGGGGCTTCA  
3251 GCTGCAGCCC AGGTGGGGCC CTGGAGCCCT CAGAACCGGA AGCAGGATTC  
3301 AAACCTCCCC TTCTCCACAG CCCCCCTGC CTCCCCAGAT GGTAGACATC  
3351 CCCCAGCTCT TACCTTCACC CTCATCTCAG AAAGGCAAGA AGCCGCCATG  
3401 TCCGCACCTT GGGGCCTGGG CTTCCCCCTC TCTGTGCCAG CGGTTCCAG  
3451 CACCTGGGGA GGGGCTGTGG CCTGACCAGA CCCCAGGCC ACCCCACATA  
3501 GTATACTAGC TGCCCACTCT GGGGCAGGAA CTGGAAAATC CATCCCTTTT  
3551 GAACAACCAC CTTCAATGAC CCCCCCATC TGGGACCAGA CTTGGTCTCT  
3601 AAGTTATTCA GCACCCCCAG TGCAGGAGGG TCCTCCCCC ACCCCCCGAA  
3651 GTCCCTGGAG CCCGGAGCAG AGCCCCACCT GTGATTCCTG GTGTTAGGGC  
3701 ACCTCAAACC TTGGGCTGGA CCACACCCCT TCCCGCCATT TCCAGACCCC  
3751 TACCTGTACT CCCAGTGCT CCCAGGGGC CTCTTGATGC TGCACGGGAC  
3801 CCTGCAGGGC TCGGTCAGTG ATGTGTTTTG TCCCAGTTA ACCGCCATCC  
3851 AGCGACCTGG TTCCAGGAGG AGCTCAGGTC ACCCCCACCA CCGCGCCAC  
3901 TCGTCTGCC GCCCTAGGCT TTCAGACATC ATTAGTCCG ACACTTGTA  
3951 AACTCCGAGA CGTGCCGTGG TCTCAGCAAT GCACCTGTTT TATACATGAT  
4001 TGTGTAATTT AAAGGTATAT AAATACAAAT ATATATATTA TATCTATATC  
4051 TATCAGTTGT GACCGTATGG CTGTCGATAA AACCAGAATT C

FIG. 46B  
SUBSTITUTE SHEET (RULE 26)



44/61

```

1   GAATTCGTCG ACCCACGGTC CGGGAAGCCT TTCACAAGCA GATGATGGGC
51  GGCTTCAAGG AGACCAAGGA AGCTGAGGAC GGCTTTCGGA AGGCACAGAA
101 GCCCTGGGCC AAGAAGCTGA AAGAGGTAGA AGCAGCAAAG AAAGCCCACC
151 ATGCAGCGTG CAAAGAGGAG AAGCTGGCTA TCTCACGAGA AGCCAACAGC
201 AAGGCAGACC CATCCCTCAA CCCTGAACAG CTCAAGAAAT TGCAAGACAA
251 AATAGAAAAG TGCAAGCAAG ATGTTCTTAA GACCAAAGAG AAGTATGAGA
301 AGTCCCTGAA GGAACCTGAC CAGGGCACAC CCCAGTACAT GGAGAACATG
351 GAGCAGGTGT TTGAGCAGTG CCAGCAGTTC GAGGAGAAAC GCCTTCGCTT
401 CTTCCGGGAG GTTCTGCTGG AGGTTTCAGAA GCACCTAGAC CTGTCCAATG
451 TGGCTGGTTA CAAAGCCATT TACCATGACC TGGAGCAGAG CATCAGAGCA
501 GCTGATGCAG TGGAGGACCT GAGGTGGTTC CGAGCCAATC ACGGGCCGGG
551 CATGGCCATG AACTGGCCGC AGTTTGAGGA GTGGTCCGCA GACCTGAATC
601 GAACCCTCAG CCGGAGAGAG AAGAAGAAGT CCACTGACGG CGTCACCCTG
651 ACGGGCATCA ACCAGACAGG CGACCACTCT CTGCCGAGTA AGCCCAGCAG
701 CACCCTTAAT GTCCCGAGCA ACCCCGCCCA GTCTGCGCAG TCACAGTCCA
751 GCTACAACCC CTTGAGGAT GAGGACGACA CGGGCAGCAC CGTCAGTGAG
801 AAGGACGACA CTAAGGCCAA AAATGTGAGC AGCTACGAGA AGACCCAGAG
851 CTATCCCACC GACTGGTCAG ACGATGAGTC TAACAACCCC TTCTCCTCCA
901 CGGATGCCAA TGGGGACTCG AATCCATTCTG ACGACGACGC CACCTCGGGG
951 ACGGAAGTGC GAGTCCGGGC CCTGTATGAC TATGAGGGGC AGGAGCATGA
1001 TGAGCTGAGC TTCAAGGCTG GGGATGAGCT GACCAAGATG GAGGACGAGG
1051 ATGAGCAGGG CTGGTGCAAG GGACGCTTGG ACAACGGGCA AGTTGGCCTA
1101 TACCCGGCAA ATTATGTGGA GGCATCCAG TGA

```

FIG. 48

```

1   RIRPTVREA FHKQMMGGFK ETKEAEDGFR KAQKPWAKKL KEVEAAKKAH
51  HAACKEEKLA ISREANSKAD PSLNPEQLKK LQDKIEKCKQ DVLKTKEKYE
101 KSLKELDQGT PQYMENMEQV FEQCQFEEK RLRFFREVLL EVQKHLDSL N
151 VAGYKAIYHD LEQSIRAADA VEDLRWFRAN HGPGMAMNWP QFEWSADLN
201 RTLSRREKKK STDGVTLTGI NQTGDQSLPS KPSSTLNVPS NPAQSAQSQS
251 SYNPFEDDD TGSTVSEKDD TKAKNVSSYE KTQSYPTDWS DDESNNPFSS
301 TDANGDSNPF DDDATSGTEV RVRALYDYE QEHDELSFKA GDELT KMEDE
351 DEQGWCKGRL DNGQVGLYPA NYVEAIQ

```

FIG. 49

SUBSTITUTE SHEET (RULE 26)

45/61

1 AAAGGGAGG AGAGTGTCAA AAAGAAGGAT  
30 GGCGAGGAAA AAGGCAAACA GGAAGCACAA GACAAGCTGG  
70 GTCGGCTTTT CCATCAACAC CAAGAACCAG CTAAGCCAGC  
110 TGTCCAGGCA CCCTGGTCCA CTGCAGAAAA AGGGTCCACT  
150 TACCATTCTT GCACAGGAAA ATGTAAAAGT GGTGTATTAC  
190 CGGGCACTGT ACCCCTTTGA ATCCAGAAGC CATGATGAAA  
230 TCACTATCCA GCCAGGAGAC ATAGTCATGG TGGATGAAAG  
270 CCAAAGTGGG GAACCCGGCT GGCTTGGAGG AGAATTAATA  
310 GGAAAGACAG GGTGGTTCCC TGCAAACTAT GCAGAGAAAA  
350 TCCCAGAAAA TGAGGTTCCC GCTCCAGTGA AACCAGTGAC  
390 TGATTCAACA TCTGCCCTG CCCCCAACT GGCCTTGCGT  
430 GAGACCCCCG CCCCTTTGGC AGTAACCTCT TCAGAGCCCT  
470 CCACGACCCC TAATACTGG GCCGACTTCA GCTCCACGTG  
510 GCCCACCAGC ACGAATGAGA AACCAGAAAC GGATAACTGG  
550 GATGCATGGG CAGCCCAGCC CTCTCTCACC GTTCCAAGTG  
590 CCGGCCAGTT AAGGCAGAGG TCCGCCTTTA CTCCAGCCAC  
630 GGCCACTGGC TCCTCCCCGT CTCCTGTGCT AGGCCAGGGT  
670 GAAAAGGTGG AGGGGCTACA AGCTCAAGCC CTATATCCTT  
710 GGAGAGCCAA AAAAGACAAC CACTTAAATT TTAACAAAAA  
750 TGATGTCATC ACCGTCCTGG AACAGCAAGA CATGTGGTGG  
790 TTTGGAGAAG TTCAAGGTCA GAAGGGTTGG TTCCCCAAGT  
830 CTTACGTGAA ACTCATTTCA GGGCCCATAA GGAAGTCTAC  
870 AAGCATGGAT TCTGGTTCTT CAGAGAGTCC TGCTAGTCTA  
910 AAGCGAGTAG CCTCTCCAGC AGCCAAGCCG GTCGTTTCGG  
950 GAGAAGAAAT TGCCCAGGTT ATTGCCTCAT ACACCGCCAC  
990 CGGCCCCGAG CAGCTCACTC TCGCCCCTGG TCAGCTGATT  
1030 TTGATCCGAA AAAAGAACCC AGGTGGATGG TGGGAAGGAG  
1070 AGCTGCAAGC ACGTGGGAAA AAGCGCCAGA TAGGCTGGTT  
1110 CCCAGCTAAT TATGTAAAGC TTCTAAGCCC TGGGACGAGC  
1150 AAAATCACTC CAACAGAGCC ACCTAAGTCA ACAGCATTAG  
1190 CGGCAGTGTG CCAGGTGATT GGGATGTACG ACTACACCGC  
1230 GCAGAATGAC GATGAGCTGG CCTTCAACAA GGGCCAGATC  
1270 ATCAACGTCC TCAACAAGGA GGACCCTGAC TGGTGGAAAG  
1310 GAGAAGTCAA TGGACAAGTG GGGCTCTTCC CATCCAATTA  
1370 TGTGAAGCTG ACCACAGACA TGGACCCAAG CCAGCAATGA

FIG. 50

SUBSTITUTE SHEET (RULE 26)

46/61

1 KGRRVSKRRM ARKKANRKHK TSWVGFSINT KNQLSQLSRH  
41 PGPLQKKGPL TISAQENVKV VYYRALYPFE SRSHDEITIQ  
81 PGDIVMVDES QTGEPGWLGG ELKGKTGWFP ANYAEKIPEN  
121 EVPAPVKPVT DSTSAPAPKL ALRETPAPLA VTSSEPSTTP  
161 NNWADFSSTW PTSTNEKPET DNWDAAWAQPSLTVPSAGQL  
201 RQRSAFTPAT ATGSSPSPVL GQGEKVEGLQ AQALYPWRAK  
241 KDNHLNFNKN DVITVLEQQD MWWFGEVQGO KGWFPKSYVK  
281 LISGPIRKST SMDSGSSESP ASLKRVASPA AKPVVSGEEI  
321 AQVIASYTAT GPEQLTLAPG QLILIRKKNP GGWWEGELQA  
361 RGKKRQIGWF PANYVKLLSP GTSKITPTEP PKSTALAAVC  
401 QVIGMYDYTA QNDELAFNK GQIINVLNKE DPDWWKGEVN  
441 GQVGLFPSNY VKLTTDMDPS QQ

FIG. 51

1 GAATTCGCGG CCGCGTCGAC CAAGATCATT CCTGGGAGTG  
41 AAGTAAAACG GGAAGAACCA GAAGCTTTGT ATGCAGCTGT  
81 AAATAAGAAA CCTACCTCGG CAGCCTATTC AGTTGGAGAA  
121 GAATATATTG CACTTTATCC ATATTCAAGT GTGGAACCTG  
161 GAGATTTGAC TTTCACAGAA GGTGAAGAAA TATTGGTGAC  
201 CCAGAAAGAT GGAGAGTGGT GGACAGGAAG TATTGGAGAT  
241 AGAAGTGGAA TTTTCCATC AAACATATGTC AAACCAAAGG  
281 ATCAAGAGAG TTTTGGGAGT GCTAGCAAGT CTGGAGCATC  
321 AAATAAAAAA CCTGAGATTG CTCAGGTAAC TTCAGCATAT  
361 GTTGCTTCTG GTTCTGAACA ACTTAGCCTT GCACCAGGAC  
401 AGTTAATATT AATTCTAAAG AAAAATACAA GTGGGTGGTG  
441 GCAAGGAGAG TTACAGGCCA GAGGAAAAAA GCGACAGAAA  
481 GGATGGTTTC CTGCCAGTCA TGTTAAACTT TTGGGTCCAA  
521 GCAGTGAAAG AGCCACACCT GCCTTTCATC CTGTATGTCA  
561 GGTGATTGCT ATGTATGACT ATGCAGCAAA TAATGAAGAT  
601 GAGCTCAGTT TCTCCAAGGG ACAACTCATT AATGTTATGA  
641 ACAAAGATGA TCCTGATTGG TGGCAAGGAG AGATCAACGG  
681 GGTGACTGGT CTCTTTCCTT CAACTACGT TAAGATGACG  
721 ACAGACTCAG ATCCAAGTCA ACAGTGA

FIG. 52

SUBSTITUTE SHEET (RULE 26)

47/61

1 EFAAASTKII PGSEVKREEP EALYAAVNKK PTSAAYSVGE  
 41 EYIALYPYSS VEPGDLTFTE GEEILVTQKD GEWWTGSIGD  
 81 RSGIFPSNYV KPKDQESFGS ASKSGASNKK PEIAQVTSAY  
 121 VASGSEQLSL APGQLILILK KNTSGWWQGE LQARGKKRQK  
 161 GWFPASHVKL LGPSSERATP AFHPVCQVIA MYDYAANNED  
 201 ELSFSKGQLI NVMNKDDPDW WQGEINGVTG LFPSNYVKMT  
 241 TSDPSQQ

## FIG. 53

HSLHLHRHQGRKERARYDLEAAQDNELTFKAGEIMTVLDDSDPNWWKGETHQGIGLFPSN 60  
 FVTADLTAEPEMIKTEKKTVOFSDDVQVETIEPEPEPAFIDEDKMDQLLQMLQSTDPSDD 120  
 QPDLPELLHLEAMCHQMGPLIDEKLEDIDRKHSELSELNKVMESLSLYTKLMNEDPMYS 180  
 MYAKLONQPYMQSSGVSQVYAGPPPSGAYLVAGNAQMSHLQSYSLPPEQLSSLQAV 240  
 VPPSANPALPSQQTQAAYPNRSPGDLMPGDSECRGSAEDSQMRISPPYFPTGQQA 296

## FIG. 55

IRGRVDQGEWPLPGRGTPGPSGLCVPEDQCRVRDLKGWLSFWAKAEKEE 50  
 ENRRLEEKRWAEAAQRQLEQERRERELREARREQRYQEQQGEASQSRT 100  
 WEQQQEVVSRNRNEQESAVHPREIFKQKERAMSTTSISSPQPGKLRSPFL 150  
 QKQLTQPETHFGREPAAAI SRPRADLPAEEPAPSTPPCLVQAEAAVYEE 200  
 PPEQETFYEQPPLVQQQGAGSEHIDHHIQGQGLSGQGLCARALYDYQAAD 250  
 DTEISFDPENLITGIEVIDEGWWRGYGPDGHFGMFPANYVELIDEAEGTS 300  
 CPSPLRHGFLIAGRGGLGVDIQHSSRNRTPSEDEASGLPPAWQTQVTPN 350  
 AAMAW 355

## FIG. 57

GRVDIERKRLELMQKKLEDEAARKAKQGKENLWKENLRKEEEEKQKRLQEEKTOEKIQE 60  
 EERKAEKQRETASVLVNYRALYPFEARNHDEMSFNSGDI IQVDEKTVGEPGWLYGSFQG 120  
 NFGWFPKNYVEKMPSSENEKAVSPKKALLPPTVSLSATSTSSSEPLSSNQPASVTDYQNV 180  
 FSNLTVNTSWQKKSFAFTRTVSPGSVSPIHGQGVVENLKAQALCSWTAKKDNHLNFSKHD 240  
 IITVLEQQENWWFGEVHGGRGWFPKSYVKIIPGSEVKREEPEALYAAVNKKPTSAAYSVG 300  
 EEYIALYPYSSVEPGDLTFTEGEEILVTQKDGEWWTGSIGDRSGIFPSNYVKPKDQESFG 360  
 SASKSGASNKKPEIAQVTSAYVASGSEQLSLAPGQLILILKKNTSGWWQGE LQARGKKRQ 420  
 KGWFPASHVKLLGPSSERATPAFHPVCQVIAMDYAANNEDELSFSKGQLINVMNKDDPD 480  
 WWQGEINGVTGLFPSNYVKMTTSDPSQQ 509

FIG. 59  
 SUBSTITUTE SHEET (RULE 26)

48/61

CACTCTCTACACTTGCACCGGCATCAAGGACGAAAAGAAC 40  
GCGCTAGATATGACTTGGAAGCTGCTCAAGACAATGAACT 80  
TACTTTCAAAGCTGGAGAAATTATGACAGTTCTTGATGAC 120  
AGTGATCCTAACTGGTGGAAAGGTGAAACCCATCAAGGCA 160  
TAGGGTTATTTCTTCTAATTTTGTGACTGCAGATCTCAC 200  
TGCTGAACCAGAAATGATTAACAGAGAAGAAGACGGTA 240  
CAATTTAGTGATGATGTTTCAGGTAGAGACAATAGAACCAG 280  
AGCCGGAACCAGCCTTTATTGATGAAGATAAAATGGACCA 320  
GTTGCTACAGATGCTGCAAAGTACAGACCCCAGTGATGAT 360  
CAGCCAGACCTACCAGAGCTGCTTCATCTTGAAGCAATGT 400  
GTCACCAGATGGGACCTCTCATTGATGAAAAGCTGGAAGA 440  
TATTGATAGAAAACATTTCAGAACTCTCAGAACTTAATGTG 480  
AAAGTGATGGAGGCCCTTTCTTATATACCAAGTTAATGA 520  
ACGAAGATCCGATGTATTCCATGTATGCAAAGTTACAGAA 560  
TCAGCCATATTATATGCAGTCATCTGGTGTTTCTGGTTCT 600  
CAGGTGTATGCAGGGCCTCCTCCAAGTGGTGCCTACCTGG 640  
TTGCAGGGAACGCGCAGATGAGCCACCTCCAGAGCTACAG 680  
TCTTCCCCCGGAGCAGCTGTCTTCTCTCAGCCAGGCAGTG 720  
GTCCCACCATCCGCAAACCCAGCCCTTCTAGTCAGCAGA 760  
CTCAGGCCGCTTACCCAAACCGCTCCCCAGGGGACCTCAT 800  
GAAGCCCGGTGATTCTGAATGCCGTGGATCTGCCGAGGAT 840  
TCCCAGATGCGTATTTCTCCTCCGTACTTCCCCACAGGAC 880  
AGCAGGCTTGAATAGCTGATTGCCTATGCAGGACAACAGG 920  
CTTGAATAGCTGACTGCCTATGCATTCTTTGCTTGCCA 960  
GTTTTTTGGACATCAAACCTTGACAGATCCAAGATTATTAC 1000  
TTTGATCTTCCCCACACCCCTCCACCCCCGAGTCTACTA 1040  
TGGTCCCATCATAGTATTCTGAAAATCAGTGAATGGCCAC 1080  
TCTACCAGTTATTTCTACCAGTTTTTAGGTTCTAAACCTC 1120  
AGGCATTCTGGACTCTTCTGTTTATTATCATATTTTGAAG 1160  
GCATTATCTTCAAATCTATCTAGACTCTGACCCTTTCTC 1200  
CCATCTCCACCATTACTGCCGTGGCTCTTCTGCTGGTCGG 1240  
CTCTCTCCTGGTGGATCCGTAATAACCTGCAGTCAGCTAT 1280  
CCTGGTCCAGAAGGGAACCCCGTTAAACCCTGTTGGAATC 1320  
TTATCACGCTTCTGCTCCAGAACGAACCCAGTCTGTCTGT 1360  
CTCACTCAGAGTGTAAGCTACAGTCCTTATTGTGGCCATC 1400  
AGGTGCTGTGTGTTCTCCAGCCCCCTCCCCACCACCGCAG 1440  
TCCTGCCGGTGATCTTAGCTGCTCTCCCCTCGGAACCCCC 1480  
TGCGGCCCCCTCTGCCGCAACAXTCGTGGCCTGCTGTTCC 1520  
TTGAACATGCTTGGTGTTTTCTCTCCTCAAAGGCTTCTT 1560  
CTGTTTACCTGAAATGTACTTGCTAGGGAAATCTTATCC 1600  
TGGCTCACTCCGCTTACTTTTTTCCACATCTTTGCTTAA 1640  
GTTATTGCCCTTATTGGAGAAGGCACCCCTACCATAAACT 1680  
AGAAATCCCTTGCCCCCAAGCTGCTCCTTT 1710

**FIG. 54**  
SUBSTITUTE SHEET (RULE 26)

49/61

GAATTCGCGGCCGCGTCGACCAAGGAGAGTGGCCGCTTCC 40  
AGGACGTGGGACCCAGGCCCCAGTGGGCTCTGTGTACCA 80  
GAAGACCAATGCCGTGTCAGAGATTTAAAGGGTTGGTTAG 120  
ACAGCTTCTGGGCCAAAGCAGAGAAGGAGGAGGAGAACCG 160  
TCGGCTGGAGGAAAAGCGGTGGGCCGAGGAGGCACAGCGG 200  
CAGCTGGAGCAGGAGCGCCGGGAGCGTGAGCTGCGTGAGG 240  
CTGCACGCCGGGAGCAGCGCTATCAGGAGCAGGGTGGCGA 280  
GGCCAGCCCCCAGAGCAGGACGTGGGAGCAGCAGCAAGAA 320  
GTGGTTTCAAGGAACCGAAATGAGCAGGAGTCTGCCGTGC 360  
ACCCGAGGGAGATTTTCAAGCAGAAGGAGAGGGCCATGTC 400  
CACCACCTCCATCTCCAGTCCTCAGCCTGGCAAGCTGAGG 440  
AGCCCCTTCTGCAGAAGCAGCTCACCAACCAGAGACCC 480  
ACTTTGGCAGAGAGCCAGCTGCTGCCATCTCAAGGCCAG 520  
GGCAGATCTCCCTGCTGAGGAGCCGGCGCCAGCACTCCT 560  
CCATGTCTGGTGCAGGCAGAAGAGGAGGCTGTGTATGAGG 600  
AACCTCCAGAGCAGGAGACCTTCTACGAGCAGCCCCACT 640  
GGTGCAGCAGCAAGGTGCTGGCTCTGAGCACATTGACCAC 680  
CACATTAGGGCCAGGGGCTCAGTGGGCAAGGGCTCTGTG 720  
CCCGTGCCCTGTACGACTACCAGGCAGCCGACGACACAGA 760  
GATCTCCTTTGACCCCGAGAACCTCATCACGGGCATCGAG 800  
GTGATCGACGAAGGCTGGTGGCGTGGCTATGGGCCGGATG 840  
GCCATTTTGGCATGTTCCCTGCCAATTACGTGGAGCTCAT 880  
TGATGAGGCTGAGGGCACATCTTGCCCTTCCCCTCTCAGA 920  
CATGGCTTCCTTATTGCTGGAAGAGGAGGCCTGGGAGTTG 960  
ACATTGAGCACTCTTCCAGGAATAGGACCCCCAGTGAGGA 1000  
TGAGGCCTCAGGGCTCCCTCCGGCTTGGCAGACTCAGCCT 1040  
GTCACCCCAAATGCAGCAATGGCCTGGTGATTCCCACACA 1080  
TCCTTCCTGCATCCCCGACCCTCCCAGACAGCTTGGCTC 1120  
TTGCCCCTGACAGGATACTGAGCCAAGCCCTGCCTGTGGC 1160  
CAAGCCCTGAGTGGCCACTGCCAAGCTGCGGGGAAGGGTC 1200  
CTGAGCAGGGGCATCTGGGAGGCTCTGGCTGCCTTCTGCA 1240  
TTTATTGCTTTTTTCTTTTTCTTTGCTTCTAAGGGGT 1280  
GGTGGCCACCACTGTTTAGAATGACCCTTGGGAACAGTGA 1320  
ACGTAGAGAATTGTTTTTAGCAGAGTTTGTGACCAAAGTC 1360  
AGAGTGGATCATGGTGGTTTGGCAGCAGGGAATTTGTCTT 1400  
GTTGGAGCCTGCTCTGTGCTCCCCACTCCATTTCTCTGTC 1440  
CCTCTGCCTGGGCTATGGGAAGTGGGGATGCAGATGGCCA 1480  
AGCTCCCACCCTGGGTATTCAAAAACGGCAGACACAACAT 1520  
GTTCTCCACGCGGCTCACTCGATGCCTGCAGGCCCCAGT 1560  
GTGTGCCTCAACTGATTCTGACTTCAGGAAAAGTAACACA 1600  
GAGTGGCCTTGGCCTGTTGTCTTCCCCTATTTTCTGTCCC 1640  
AGCTCATCCGTGGTGAAGCGCCCGCGAATTCCAGCTGAG 1680  
CGGCCGC 1687

**FIG. 56**  
**SUBSTITUTE SHEET (RULE 26)**

50/61

GCGGCCGCGTCGACATTGAAAGGAAAAGATTAGAACTAAT 40  
GCAGAAAAAGAACTAGAAGATGAGGCTGCAAGGAAAGCA 80  
AAGCAAGGAAAAGAAAACCTTATGGAAAGAAAATCTTAGAA 120  
AGGAGGAAGAAGAAAAACAAAAGCGACTCCAGGAAGAAAA 160  
AACACAAGAAAAAATTCAAGAAGAGGAACGGAAAGCTGAG 200  
GAGAAACAACGTGAGACAGCTAGTGT TTTTGGTGAATTATA 240  
GAGCATTATACCCCTTTGAAGCAAGGAACCATGATGAGAT 280  
GAGTTTTAATTCTGGAGATATAATTCAGGTTGATGAAAAA 320  
ACCGTAGGAGAACCTGGTTGGCTTTATGGTAGTTTTCAAG 360  
GAAATTTTGGCTGGTTTCCATGCAATTATGTAGAAAAAAT 400  
GCCATCAAGTGAAAATGAAAAAGCTGTATCTCCAAAGAAG 440  
GCCTTACTTCCTCCTACAGTTTCTTTATCTGCTACCTCAA 480  
CTTCCTCTGAACCACTTTCTTCAAATCAACCAGCATCAGT 520  
GACTGATTATCAAAATGTATCTTTTTCAAACCTAACTGTA 560  
AATACATCATGGCAGAAAAAATCAGCCTTCACTCGAACTG 600  
TGTCCCCTGGATCTGTATCACCTATTCATGGACAGGGACA 640  
AGTGGTAGAAAACCTTAAAAGCACAGGCCCTTTGTTCTGG 680  
ACTGCAAAGAAAGATAACCACTTGAACCTCTCAAAACATG 720  
ACATTATTACTGTCTTGGAGCAGCAAGAAAATTGGTGGTT 760  
TGGGGAGGTGCATGGAGGAAGAGGATGGTTTCCCAAATCT 800  
TATGTCAAGATCATTCTGGGAGTGAAGTAAACGGGAAG 840  
AACCAGAAGCTTTGTATGCAGCTGTAAATAAGAAACCTAC 880  
CTCGGCAGCCTATTCAAGTTGGAGAAGAATATATTGCACTT 920  
TATCCATATTCAAGTGTGGAACCTGGAGATTTGACTTTCA 960  
CAGAAGGTGAAGAAATATTGGTGACCCAGAAAGATGGAGA 1000  
GTGGTGGACAGGAAGTATTGGAGATAGAAGTGAATTTTT 1040  
CCATCAAACCTATGTCAAACCAAAGGATCAAGAGAGTTTTG 1080  
GGAGTGCTAGCAAGTCTGGAGCATCAAATAAAAAACCTGA 1120  
GATTGCTCAGGTAACCTTCAGCATATGTTGCTTCTGGTTCT 1160  
GAACAACTTAGCCTTGCAACAGGACAGTTAATATTAATTC 1200  
TAAAGAAAAATACAAGTGGGTGGTGGCAAGGAGAGTTACA 1240  
GGCCAGAGGAAAAAAGCGACAGAAAGGATGGTTTCTGCC 1280  
AGTCATGTTAAACTTTTGGGTCCAAGTAGTGAAAGAGCCA 1320  
CACCTGCCTTTCATCCTGTATGTCAGGTGATTGCTATGTA 1360  
TGACTATGCAGCAAATAATGAAGATGAGCTCAGTTTCTCC 1400  
AAGGGACAACCTCATTAAATGTTATGAACAAAGATGATCCTG 1440  
ATTGGTGGCAAGGAGAGATCAACGGGGTGAAGTGGTCTCTT 1480  
TCCTTCAAACCTACGTTAAGATGACGACAGACTCAGATCCA 1520  
AGTCAACAGTGACCCAATGTTGTCTTCCAGTTGTGAAAGC 1560  
ACCCAGAGACCCACTATCCAAGTTTCACTCTAGCGTGGA 1600  
GGCAGGGCAGGCAGCCCTGATCAAATATCTGCTACACAAT 1640  
TCGTTTACTTCGTTTGAATGTTAGAGCCACTTGTGATTAT 1680  
TTTTTTGTGTTTCTAACTTACAGTTTAAATTTATTTGTAA 1720

**FIG. 58A**  
**SUBSTITUTE SHEET (RULE 26)**

51/61

AAAGTTAAAGGATAGTGGGTCTTTGTGTGGCTTTCCCTGC 1760  
TGTTCACTCTGGCATCTTTAGCATTTTTCTTCTTTTTTAA 1800  
TTTGATAATTGTAGGTCATTAGCATGCATATTGAGTTTGC 1840  
CCTTATGTGGTGGGAGTTCAAACACACAAAGACCCACTAT 1880  
TTGCACAACTATTCTTACTGGTTTGAATAGGCTGCCAT 1920  
GCTTTTTTAATGTTATTGCAACATGTGTATTCATTTACAG 1960  
AATTCAGATAAAATTTGCTTATGTTCTGCTATTATGTTTG 2000  
ATCTAATCCTAATCACAGTGAGCTCTTAATTAGCTCAATA 2040  
TGTGGTTTGGCCTCAAGTGTGCACTGTTTATTACTTTGTA 2080  
ATATGCCACTATGAGTACTGACATTTAGATATGTTTAAAG 2120  
GCCAAGAACTGGAAACAGCCATGCCCTGTTTTCTGTGTAT 2160  
TTGGGATGGGAATAACAACATTTTGGGGGGAGCTTTTTAA 2200  
ATCTCAGAGAAGAGGAAAGTGGCCTGCTCTGGCAGGTATG 2240  
TGCAGTGTTTCATTTGTTCCAGTCCCAAGAATGAGCACTG 2280  
TCCTATGGTAGTTTCGCTTAGGATCTTTATGTGCTCTGGGC 2320  
TAATGAAGGTACTGCATCATGTGCTGCAGCGTGTGTATTC 2360  
TTTTTCGATGACCTATAAAGGGATTATTTTTGAGGAATGA 2400  
AAGGCTCCCATCATTGACTGTGAGATGGGAAAAACCTTTC 2440  
CTAGCTTAGAGCATTTATATCTTAATCCATTTTAAAGTCA 2480  
GAGTTCATTGTTACCTGTTTTAATCAGGTGACTACATGTC 2520  
CCAGTATACAAAGGGGCACTGGTTGACATTCTTCTTAATG 2560  
TATTTAGTAAATATCATAAGAAATCCTTTAAGAGTTTAAA 2600  
TGTCCCCAAAACAGACATGCGGGCTCTAGTCAAGAATGAA 2640  
TTAGAGTGAAGGAAAGCTGTGTAACACCTGGCATTCTCT 2680  
GTGTTTCATGGAGCTTCTTTGAGGCTCTAAGATTGATTTTA 2720  
CCATCAGACTTCTCTAATACCTGTTCTTCAACCATATTGG 2760  
CTACTTTGACATAAGAATTTACTTCTTTTCCTGGAATGGA 2800  
AAACACTTTAAAAAATAATAACAAACATTATTATAAACTA 2840  
ATATATGTGAGAGGTCGACGCGGCCGCGAATTC 2873

FIG. 58B

SUBSTITUTE SHEET (RULE 26)



52/61

GAATTCGTCGACCCACGCGTCCGAAATATAACTGAAGTTGGGGCACCTAC 50  
TGAAGAAGAGGAAGAAAGTGAAAGTGAAGATAGTGAAGACAGTGGTGGGG 100  
AGGAAGAAGATGCAGAGGAGGAAGAGGAAGAGAAAGAGGAAAATGAATCT 150  
CACAAATGGTCAACCGGTGAAGAATACATCGCTGTTGGAGATTTTACTGC 200  
TCAGCAAGTTGGAGATCTTACATTTAAGAAAGGGGAAATTCTCCTTGTA 250  
TTGAAAAAAACCTGATGGTTGGTGGATAGCTAAGGATGCCAAAGGAAAT 300  
GAAGGTCTTGTTCCAGAACCTACCTAGAGCCTTATAGTGAAGAAGAAGA 350  
AGGCCAAGAGTCAAGTGAAGAGGGCAGTGAAGAAGATGTAGAGGCGGTGG 400  
ATGAAACAGCAGATGGAGCAGAAGTTAAGCAAAGAACTGATCCCCACTGG 450  
AGTGCTGTTTCAGAAAGCGATTTTCAGAGGCGGGCATCTTCTGTCTTGTTAA 500  
TCATGTCTCGTTTTGCTACCTAATAGTTCTGATCCGTCCCTAA 543

## FIG. 60

GAATTCGGCGGACTTCGCGGCCGCGTCGACGAAGAAACCT 40  
GAAGGACACACTAGGCCTCGGCAAGACGCGCAGGAAGACC 80  
AGCGCGCGGGATGCGTCCCCACGCCAGCACGGACGCCG 120  
AGTACCCCGCCAATGGCAGCGGCGCCGACCGCATCTACGA 160  
CCTCAACATCCCGGCCTTCGTCAAGTTCGCTATGTGGCC 200  
GAGCGGGAGGATGAGTTGTCCCTGGTGAAGGGGTGCGCG 240  
TCACCGTCATGGAGAAGTGCAGCGACGGTTGGTGGCGGGG 280  
CAGCTACAACGGGCAGATCGGCTGGTTCCCTCCAACCTAC 320  
GTCTTGGAGGAGGTGGACGAGGCGGTTGCGGAGTCCCCAA 360  
GCTTCCTGAGCCTGCGCAAGGGCGCCTCGCTGAGCAATGG 400  
CCAGGGCTCCCGCGTGCTGCATGTGGTCCAGACGCTGTAC 440  
CCCTTCAGCTCAGTCACCGAGGAGGAGCTCAACTTCGAGA 480  
AGGGGGAGACCATGGAGGTGATTGAGAAGCCGGAGAACGA 520  
CCCCGAGTGGTGGAAATGCAAAAATGCCCGGGGCCAGGTG 560  
GGCCTCGTCCCCAAAACTACGTGGTGGTCTCAGTGACG 600  
GGCCTGCCCTGCACCCTGCGCACGCCCCACAGATAAGCTA 640  
CACCGGGCCCTCGTCCAGCGGGCGCTTCGCGGGCAGAGAG 680  
TGGTACTACGGGAACGTGACGCGGCACCAGGCCGAGTGCG 720  
CCCTCAACGAGCGGGGCGTGGAGGGCGACTTCCTCATTAG 760  
GGACAGCGAGTCCTCGCCAGCGACTTCTCCGTGTCCCTT 800  
AAAGCGTCAGGGAAGAACAACACTTCAAGGTGCAGCTCG 840  
TGGACAATGTCTACTGCATTGGGCAGCGGCGCTTCCACAC 880  
CATGGACGAGCTGGTGGAACTACAAAAAGGCGCCCATC 920  
TTCACCAGCGAGCACGGGGAGAAGCTCTACCTCGTCAGGG 960  
CCCTGCAGTGA 971

## FIG. 62

SUBSTITUTE SHEET (RULE 26)

53/61

GAATTCGTCGACCCACGCGTCCGAAATATAACTGAAGTTGGGGCACCTACTGAAGAAGAGGAAGAAAGTG  
+++++ 70  
E F V D P R V R N I T E V G A P T E E E E E S 23  
AAAGTGAAGATAGTGAAGACAGTGGTGGGGAGGAAGAAGATGCAGAGGAGGAAGAGGAAGAGAAAGAGGA  
+++++ 140  
E S E D S E D S G G E E E D A E E E E E E K E E 47  
AAATGAATCTCACAAATGGTCAACCGGTGAAGAATACATCGCTGTTGGAGATTTTACTGCTCAGCAAGTT  
+++++ 210  
N E S H K W S T G E E Y I A V [G D F T A Q Q V 70  
SH3  
GGAGATCTTACATTTAAGAAAGGGGAAATTCTCCTTGTAATTGAAAAAACCTGATGGTTGGTGGATAG  
+++++ 280  
G D L T F K K G E I L L V I E K K P D G W W I 93  
CTAAGGATGCCAAAGGAAATGAAGGTCTTGTTCCCAGAACCTACCTAGAGCCTTATAGTGAAGAAGAAGA  
+++++ 350  
A K D A K G N E G L V P R T Y] L E P Y S E E E E 117  
AGGCCAAGAGTCAAGTGAAGAGGGCAGTGAAGAAGATGTAGAGCGGTGGATGAAACAGCAGATGGAGCA  
+++++ 420  
G Q E S S E E G S E E D V E A V D E T A D G A 140  
GAAGTTAAGCAAAGAACTGATCCCCACTGGAGTGCTGTTTCAGAAAGCGATTTCAGAGGCGGCATCTTT  
+++++ 490  
E V K Q R T D P H W S A V Q K A I S E A G I F 163

FIG.61A

SUBSTITUTE SHEET (RULE 26)

54/61

---

GTCTTGTTAATCATGTCTCGTTTTGCTACCTAATAGTTCTGATCCGTCCCTAA  
+ + + + +  
C L V N H V S F C Y L I V L I R P 180  
+ + + + +

FIG.61B

SUBSTITUTE SHEET (RULE 26)

55/61

GAATTCGGCGGACTTCGCGGCCGCTCGACGAAGAAACCTGAAGGACACACTAGGCCTCGGCAAGACGCG	70
I R R T S R P R R R R N L K D T L G L G K T R	23
CAGGAAGACCAGCGCGGGGATGCGTCCCCACGCCACGACGCGCGAGTACCCGCCAATGGCAGC	140
R K T S A R D A S P T P S T D A E Y P A N G S	46
GGCGCCGACCGCATCTACGACCTCAACATCCCGGCCCTTCGTCAAGTTCGCCTATGTGGCCGAGCGGGAGG	210
G A D R I Y D L N I P A F V K [F A Y V A E R E	69
ATGAGTTGTCCCTGGTGAAGGGGTGCGCGTCACCGTCATGGAGAAGTGCAGCGACGGTTGGTGGCGGGG	280
D E L S L V K G S R V T V M E K C S D G W W R G	93
CAGCTACAACGGCCAGATCGGCTGGTTCCCTCCAACCTACGTCTTGGAGGAGGTGGACGAGGCGGTTGCG	350
S Y N G Q I G W F P S N Y] V L E E V D E A V A	116
GAGTCCCCAAGCTTCCTGAGCCTGCGCAAGGGCGCCTCGCTRGAGCAATGGCCAGGGCTCCCGCGTGCTGC	420
E S P S F L S L R K G A S L S N G Q G S R V L	139
ATGTGGTCCAGACGCTGTACCCCTTCAGCTCAGTCACCGAGGAGGAGCTCAACTTCGAGAAGGGGGAGCAC	490
H V V Q T L [Y P F S S V T E E E L N F E K G E T	163

FIG.63A  
SUBSTITUTE SHEET (RULE 26)

56/61

CATGGAGGTGATTGAGAAGCCGGAGAACGACCCCGAGTGGTGGAAATGCAAAAATGCCCGGGGCCAGGTG  
|-----| 560  
M E V I E K P E N D P E W W K C K N A R G Q V 186  
|-----|  
GGCCTCGTCCCCAAAACACTACGTGGTGGTCCTCAGTGACGGGCCTGCCCTGCACCCTGCGCAGCCCCAC  
|-----| 630  
G L V P K N Y] V V V L S D G P A L H P A H A P 209  
|-----|  
AGATAAGCTACACCGGGCCCTCGTCCAGCGGGCGCTTCGCGGGCAGAGAGTGGTACTACGGGAACGTGAC  
|-----| 700  
Q I S Y T G P S S S G R F A G R E W Y Y G N V T 233  
|-----|  
GCGGCACCAGGCCGAGTGCGCCCTCAACGAGCGGGCGTGGAGGGCGACTTCCTCATTAGGGACAGCGAG  
|-----| 770  
R H Q A E C A L N E R G V E G D F L I R D S E 256  
|-----|  
TCCTCGCCCAGCGACTTCTCCGTGTCCCTTAAAGCGTCAGGGAAGAACAACACTTCAAGGTGCAGCTCG  
|-----| 840  
S S P S D F S V S L K A S G K N K H F K V Q L 279  
|-----|  
TGGACAATGTCTACTGCATTGGGCAGCGCGCTTCCACACCATGGAGGAGCTGGTGGAACTACAAAAA  
|-----| 910  
V D N V Y C I G Q R R F M T M D E L V E H Y K K 303  
|-----|  
GGCGCCCATCTTCACCAGCGAGCACGGGGAGAAGCTCTACCTCGTCAGGGCCCTGCAGTGACGGCGCCCC  
|-----| 980  
A P I F T S E H G E K L Y L V R A L Q / 322  
|-----|

FIG.63B

SUBSTITUTE SHEET (RULE 26)

57/61

GAATTCGCGGACTTCGCGGCCGCGTCGACACCAGTGCAGG 40  
TTTTGGAATATGGAGAAGCTATTGCTAAGTTTAACTTTAA 80  
TGGTGATACACAAGTAGAAATGTCCTTCAGAAAGGGTGAG 120  
AGGATCACACTGCTCCGGCAGGTAGATGAGAACTGGTACG 160  
AAGGGAGGATCCCGGGGACATCCCGACAAGGCATCTTCCC 200  
CATCACCTACGTGGATGTGATCAAGCGACCACTGGTGAAA 240  
AACCTGTGGATTACATGGACCTGCCTTTCTCCTCCTCCC 280  
CAAGTCGCAGTGCCACTGCAAGCCACAGCAACCTCAAGC 320  
CCAGCAGCGAAGAGTCACCCCGACAGGAGTCAAACCTCA 360  
CAAGATTTATTTAGCTATCAAGCATTATATAGCTATATAC 400  
CACAGAATGATGATGAGTTGGAACCTCCGCGATGGAGATAT 440  
CGTTGATGTCATGGAAAAATGTGACGATGGATGGTTTGTT 480  
GGTACTTCAAGAAGGACAAAGCAGTTTGGTACTTTTCCAG 520  
GCAACTATGTAAAACCTTTGTATCTATAA

FIG. 64

SUBSTITUTE SHEET (RULE 26)

58/61

GAATTCGCGGACTTCGCGGCCGCTCGACACCAGTGCAGGTTTTGGAATATGGAGAAGCTATTGCTAAGT 70  
CTTAAGCGCCTGAAGCGCCGCGCAGCTGTGGTCACGTCCAAAACCTTATACCTCTTCGATAACGATTCA  
ORF  
(E) F A D F A A A S T P V Q V L E Y G E A I A K 23  
TTAACTTTAATGGTGATACACAAGTAGAAATGTCCTTCAGAAAGGGTGAGAGGATCACACTGCTCCGGCA 140  
AATTGAAATTACCACTATGTGTTTCATCTTTACAGGAAGTCTTTCCCACTCTCCTAGTGTGACGAGGCCGT  
F N F N G D T Q V E M S F R K G E R I T L L R Q 47  
GGTAGATGAGAACTGGTACGAAGGGAGGATCCCGGGGACATCCCGACAAGGCATCTTCCCCATCACCTAC 210  
XXATCTACTCTTGACCATGCTTCCCTCCTAGGGCCCCTGTAGGGCTGTTCCGTAGAAGGGGTAGTGGATG  
V D E N W Y E G R I P G T S R Q G I F P I T Y 70  
GTGGATGTGATCAAGCGACCACTGGTGAAAAACCCTGTGGATTACATGGACCTGCCTTTCTCCTCCTCCC 280  
CACCTACACTAGTTCGCTGGTGACCACTTTTGGGACACCTAATGTACCTGGACGGAAGAGGAGGAGGG  
V D V I K R P L V K N P V D Y M D L P F S S S 93  
CAAGTCGCAGTGCCACTGCAAGCCCACAGCAACCTCAAGCCCAGCAGCGAAGAGTCACCCCCGACAGGAG  
GTTCAGCGTCACGGTGACGTTCGGGTGTGCTGGAGTTCGGGTCGTCGCTTCTCAGTGGGGGCTGTCTC 350  
P S R S A T A S P Q Q P Q A Q Q R R V T P Q R S 117

FIG.65A  
SUBSTITUTE SHEET (RULE, 26)

59/61

TCAAACCTCACAAGATTTATTTAGCTATCAAGCATTATATAGCTATATACCACAGAATGATGATGAGTTG 420  
AGTTTGGAGTGTTCTAAATAAATCGATAGTTCGTAATATATCGATATATGGTGTCTTACTACTACTCAAC  
SH3  
Q T S Q D L F S Y Q A L [Y S Y] I P Q N D [D E L] 140  
GAACTCCGCGATGGAGATATCGTTGATGTCATGGAAAAATGTGACGATGGATGGTTTGTGGTACTTCAA 490  
CTTGAGGCGCTACCTCTATAGCAACTACAGTACCTTTTTTACACTGCTACCTACCAAACAACCATGAAGTT  
F  
E L R D G D I V D V M E K C D D G [W F] V G T S 163  
GAAGGACAAAGCAGTTTGGTACTTTTCCAGGCAACTATGTAAACCTTTGTATCTATAAGAAGACTGAAA 560  
CTTCCTGTTTCGTCAAACCATGAAAAGGTCCGTTGATACATTTTGGAAACATAGATATTCTTCTGACTTT  
STOP  
R R T K Q F G T F [R G N Y] V K P L Y L ( ) 181

FIG.65B

SUBSTITUTE SHEET (RULE 26)



60/61

AATTCAAGCGCGGGTCTTTAGGATTTGCAGCTCCAGGAAGCGAGATGTCGAAGCCGCCACCCAAACCA  
+++++ 70  
N S S A G S L G F A A P G S E M S K P P P K P  
+++++  
GTCAAACCAGGGCAAGTTAAAGTCTTCAGAGCCCTGTATACGTTTGAACCCAGAACTCCAGATGAATTAT  
+++++ 140  
V K P G Q V K V F R A L Y T F E P R T P D E L  
+++++  
ACTTTGAGGAAGGTGATATTATCTACATTACTGACATGAGCGATACCAATTGGTGGAAAGGCACCTCCAA  
+++++ 210  
Y F E E G D I I Y I T D M S D T N W W K G T S K  
+++++  
AGGCAGGACTGGACTAATTCCAAGCAACTATGTGGCTGAGCAGGCAGAATCCATTGACAATCCATTGCAT  
+++++ 280  
G R T G L I P S N Y V A E Q A E S I D N P L H  
+++++  
GAAGCAGCAAAAAGAGGCAACTTGAGCTGGTTGAGAGAGTGTTTGGACAACAGAGTGGGTGTTAATGGCT  
+++++ 350  
E A A K R G N L S W L R E C L D N R V G V N G  
+++++  
TAGACAAAGCTGGAAGCACTGCCTTATACTGGGCTTGCCACGGGGGCCACAAAGATATAGTGGAATGCT  
+++++ 420  
L D K A G S T A L Y W A C H G G H K D I V E M L  
+++++  
ATTACTCAACCAAATATTGAACTGAACCAGCAGAACAAGTTGGGAGATACAGCTTTGCATGCTGCTGCC  
+++++ 490  
F T Q P N I E L N Q D N K L G D T A L H A A A  
+++++

FIG.66A

SUBSTITUTE SHEET (RULE 26)

61/61

TGGAAGGGTTATGCAGATATCGTCCAGTTGCTTCTGGCAAAAGGTGCTAGAACAGACTTAAGAAACATTG  
|-----| 560  
W K G Y A D I V Q L L L A K G A R T D L R N I  
|-----|

AGAAGAAGCTGGCCTTCGACATGGCTACCAATGCTGCCTGTGCATCTCTCCTGAAAAAGAAACAGGGAAC  
|-----| 630  
E K K L A F D M A T N A A C A S L L K K K Q G T  
|-----|

AGATGCAGTTCGAACATTAAGCAATGCCGAGGACTATCTCGATGATGAAGACTCAGATTAA  
|-----|  
D A V R T L S N A E D Y L D D E D S D <sup>STOP</sup>  
|-----|

FIG.66B

SUBSTITUTE SHEET (RULE 26)

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US96/04454

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C12Q 1/68; C12N 5/10, 15/12; C07K 14/00, 16/18; G01N 33/53

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 7.1, 7.5, 172.1, 240.1, 320.1; 530/300, 350, 387.9; 536/23.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
Please See Extra Sheet.

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	SPARKS et al. Cloning of ligand targets: Systematic isolation of SH3 domain-containing proteins. Nature Biotechnology. June 1996, Vol. 14, pages 741-744, see entire article.	1-102
X, P ---- Y	WO 95/24419 (ARIAD PHARMACEUTICALS, INC.) 14 September 1995, see pages 2-4, 13, 14, 16-18, 27 and 31, especially page 14, lines 16-22	53-95, 97-102 ----- 1-52, 96
X --- Y	SPARKS et al. Identification and Characterization of Src SH3 Ligands from Phage-displayed Random Peptide Libraries. The Journal of Biological Chemistry. September 1994, Vol. 269, No. 39, pages 23853-23856, see the abstract and page 23855, column 2.	53-95, 97, 11-102 ----- 1-52, 96

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	* T	later documents published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
* A* document defining the general state of the art which is not considered to be of particular relevance	* X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
* E* earlier documents published on or after the international filing date	* Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
* L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	* Z*	document member of the same patent family
* O* document referring to an oral disclosure, use, exhibition or other means		
* P* document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

20 JUNE 1996

Date of mailing of the international search report

15 JUL 1996

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

LORA M. GREEN

Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)\*

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US96/04454

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X — Y	RICKLES et al. Identification of Src, Fyn, Lyn, PI3K and Abl SH3 domain ligands using phage display libraries. The EMBO Journal. 1994, Vol. 12, No. 23, pp5598-5604, see abstract and page 5602, column 2.	53-95, 107-102 — 1-52, 96
Y	WO 93/18054 (N.V. INNOGENETICS) 16 September 1993, see pages 5, 24 and 25	1-52, 96

Form PCT/ISA/210 (continuation of second sheet)(July 1992)\*

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US96/04454

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US96/04454

## A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

435/6, 7.1, 7.5, 172.1, 240.1, 320.1; 530/300, 350, 387.9; 536/23.5

## B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

APS, STN, DIALOG

search terms: library, gene expression, peptide, avidin, biotin, multiple antigen peptide, phage display, antibody, SH3, SH2, zinc finger, leucine zipper

## BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s) 1-52, 69-73, 89, 90 and 94-97 drawn to methods of identifying a polypeptide comprising a functional domain of interest.

Group II, claim(s) 53-68, 74, 75, 79, 80 and 101-102, drawn to a purified polypeptide, kits containing said purified polypeptide and methods of screening for a potential drug candidate.

Group III, claim(s) 76-78, 81-88 and 100 drawn to DNA encoding a polypeptide, a vector comprising said DNA, a recombinant cell and methods of producing a fusion protein.

Group IV, claim(s) 91-93, drawn to a method of determining the potential pharmacological activities of a molecule.

Group V, claim(s) 98 and 99, drawn to an antibody.

The inventions listed as Groups I-V do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The invention of Group I is drawn to a method of identifying a polypeptide comprising a functional domain of interest, and as claimed, does not require the products of Groups II, III and V. The polypeptide and kits of Group II have a defined seq. ID, which are not required in the method of Group I. In addition, functional domains such as SH3 domains are known in the art (see for Example, Cheadle et al., J. Biol. Chem. Vol. 269, No. 39; pages 24034-24039 (1994)). Further, the method of Group IV also lacks the technical feature of Group I as Group IV does not require the use of a multivalent recognition unit complex.

The invention of Group IV also does not have the same technical features as Groups II, III and V, as the method of Group IV as claimed does not require the products of Groups II, III and V. The polypeptide and kits of Group II have a defined seq. ID, which are not required in the method of Group IV. In addition, as stated above, functional domains such as SH3 domains are known in the art.

Groups II and III also lack a single concept. Group II is drawn to polypeptide and Group III is drawn to DNA, and thus have different structure and function. In addition, as stated above, polypeptides comprising functional domains such as SH3 domains are known in the art. Group V also does not relate to a single inventive concept, as Group V is drawn to an antibody, and is not required by the method of Groups I or IV, and is a separate product than the products of Groups II and III, having a different function and structure.